ISSN 2150-5330 (online)

World Journal of *Gastrointestinal Pathophysiology*

World J Gastrointest Pathophysiol 2018 February 15; 9(1): 1-36

Published by Baishideng Publishing Group Inc

WJGP

World Journal of Gastrointestinal Pathophysiology

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World Journal of Gastrointestinal Pathophysiology
Volume 9 Number 1 February 15, 2018

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NAME OF JOURNAL
World Journal of Gastrointestinal Pathophysiology

ISSN
ISSN 2150-5330 (online)

LAUNCH DATE
April 15, 2010

Frequency
Quarterly

EDITORIAL OFFICE
Xiu-Xia Song, Director
World Journal of Gastrointestinal Pathophysiology
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
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PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive,
Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
February 15, 2018

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: http://www.f6publishing.com

World J Gastrointest Pathophysiol 2018 February 15; 9(1): 1-7

DOI: 10.4291/wjgp.v9.i1.1

ISSN 2150-5330 (online)

MINIREVIEWS

Acute cholangitis - an update

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Author contributions: Ahmed M solely contributed to this work.

Conflict-of-interest statement: None to declare.

Manuscript source: Invited manuscript

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Received: April 6, 2017
Peer-review started: April 10, 2017
First decision: May 26, 2017
Revised: July 5, 2017
Accepted: October 30, 2017
Article in press: October 30, 2017
Published online: February 15, 2018

Abstract

Acute cholangitis is bacterial infection of the extra-hepatic biliary system. As it is caused by gallstones blocking the common bile duct in most of the cases, its prevalence is greater in ethnicities with high prevalence of gallstones. Biliary obstruction of any cause is the main predisposing factor. Diagnosis is established by the presence of clinical features, laboratory results and imaging studies. The treatment modalities include administration of intravenous fluid, antibiotics, and drainage of the bile duct. The outcome is good if the treatment is started early, otherwise it could be grave.

Key words: Acute cholangitis; Ascending cholangitis; Biliary infection; Hepatic fever; Infection of the bile duct

Core tip: Acute cholangitis is a serious medical problem unless treated early. High clinical suspicion is essential to diagnose this condition. The different diagnostic criteria, treatment options, including different modalities of biliary drainage, and prognosis are described in this article.

Ahmed M. Acute cholangitis - an update. *World J Gastrointest Pathophysiol* 2018; 9(1): 1-7 Available from: URL: http://www.wjgnet.com/2150-5330/full/v9/i1/1.htm DOI: http://dx.doi.org/10.4291/wjgp.v9.i1.1

INTRODUCTION

Acute cholangitis is a clinical entity caused by bacterial infection of the biliary system, most commonly secondary to partial or complete obstruction of the bile duct or hepatic ducts. The diagnosis is established by the characteristic clinical symptoms and signs of infection, abnormal laboratory studies suggestive of infection and biliary obstruction, and abnormal imaging studies suggestive of biliary obstruction[1]. The main importance of this condition is that it is a very treatable condition if treated appropriately, but the mortality

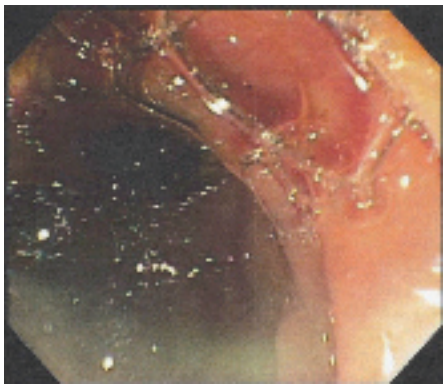

Figure 1 Pus seen extruding from the ampulla of Vater.

can be high if there is delay in treatment. There are other varieties of cholangitis, which include primary biliary cholangitis, primary sclerosing cholangitis, IgG4-related autoimmune cholangitis and recurrent pyogenic cholangitis or Oriental cholangiohepatitis[2]. We will be exclusively discussing here acute bacterial cholangitis, also called ascending cholangitis. The term ascending cholangitis comes from the migration of bacteria from the duodenum into the common bile duct. But, rarely, translocation of bacteria from the portal vein into the bile duct can also occur.

ETIOLOGY

The biliary obstruction is most commonly caused by choledocholithiasis. Other causes of obstruction include benign or malignant stricture of the bile duct or hepatic ducts, pancreatic cancer, ampullary adenoma or cancer, porta hepatis tumor or metastasis, biliary stent obstruction (due to microbial biofilm formation, biliary sludge deposition and duodenal reflux of food content), primary sclerosing cholangitis, amyloid deposition in the biliary system[3], Mirizzi syndrome (gallstone impacted in cystic duct or neck of the gall bladder causing compression on common bile duct or common hepatic duct), Lemmel's syndrome (peri-ampullary diverticulum causing distal biliary obstruction), round worm (*Ascaris lumbricoides*) or tapeworm (*Taenia saginata*) infestation of the bile duct[4], acquired immunodeficiency syndrome (commonly known as AIDS) cholangiopathy and strictured bilioenteric anastomoses[5]. Choledochocele and narrow-caliber bile duct are other risk factors for acute cholangitis. Recently, there was an outbreak of cholangitis due to carbapenem-resistant Enterobacteriaceae (CRE) as a result of exposure to contaminated duodenoscope[6]. Post-endoscopic retrograde cholangiopancreatography (ERCP) acute cholangitis can occur in 0.5% to 2.4% cases (Figure 1)[7]. As cholelithiasis is the most important risk factor, the same risk factors may play important roles in the development of acute cholangitis, particularly high fat (triglyceride) intake, sedentary life styles, obesity and rapid weight loss. Heavy alcohol consumption may lead to cirrhosis of the liver, which is a risk factor for gallstone formation.

EPIDEMIOLOGY

The prevalence of cholelithiasis varies in different ethnicities. Gallstones are found in 10% to 15% of the white population in the United States. It is much more prevalent in native Americans (60%-70%) and Hispanics but less common in Asians and African Americans[8]. Many patients get admitted to the hospital with gallstone disease and 6% to 9% of them are diagnosed with acute cholangitis[9]. Males and females are equally affected. The average age of patients presenting with acute cholangitis is 50 to 60 years. Less than 200000 cases of cholangitis occur per year in the United States.

PATHOPHYSIOLOGY

Biliary obstruction is an important factor in the pathogenesis of cholangitis. When bile flow occurs, presence of bacteria in the bile is not that significant because bacterial concentration does not increase and the intraductal pressure does not increase. Normally, there are different defensive mechanisms to prevent cholangitis. The bile salts have bacteriostatic activity and the biliary epithelium secretes IgA and mucous which probably act as anti-adherent factors. Kupffer cells on the biliary epithelium and the tight junction between the cholangiocytes prevent translocation of bacteria from the hepatobiliary system into the portal venous system. Normal bile flow flushes out any bacteria into the duodenum.

The sphincter of Oddi also prevents any migration of bacteria from the duodenum into the biliary system. In case of biliary obstruction, bile becomes stagnant in the biliary system, the intraductal pressure increases, the tight junction between cholangiocytes widen, Kupffer cells malfunction and the production of IgA is decreased[10]. "Choledochal pressure" plays an important role in the pathogenesis of acute cholangitis. The normal biliary ductal pressure is 7 to 14 cm of water (H_2O). When the intraductal pressure exceeds 25 cm of H_2O, cholangiovenous and cholangiolymphatic reflux can occur, leading to bacteremia and endotoxinemia[11]. Besides this, systemic release of inflammatory mediators like tumor necrosis factor (TNF), soluble TNF receptors, interleukin (IL)-1, IL-6 and IL-10 leads to profound hemodynamic compromise.

The most frequently found pathogens isolated in acute cholangitis are coliform organisms[12,13]. These include *Escherechia coli* (25%-50%), *Klebsiella species* (15%-20%), *Enterococcus* species (10%-20%) and *Enterobacter* species (5%-10%). Sometimes, anaerobic bacteria like Bacteroids fragilis and Clostridium perfringens can also cause acute cholangitis, particularly in patients with previous biliary surgery and in the elderly population[14]. Parasitic infestation

of the biliary system by the liver flukes *Clonorchis sinensis*, *Opisthorchis viverrini* and *Opisthorchis felineus* and the roundworm *Ascaris lumbricoides* may lead to cholangitis[15].

CLINICAL PRESENTATION

The presentation depends on the severity of cholangitis. Classically, patients present with high fever persisting for more than 24 h, abdominal pain and jaundice (Charcot's triad or hepatic fever). The right upper quadrant abdominal pain is generally mild. When the cholangitis becomes more severe, patients become hypotensive and confused (Reynold's pentad). Charcot's triad has low sensitivity (26.4%) and high specificity (95.9%). Although the presence of Charcot's triad is suggestive of acute cholangitis, it is not diagnostic. Charcot's triad is present in 26.4% to 72% of patients with acute cholangitis[16].

To improve the sensitivity of Charcot's triad, TG07 diagnostic criteria for acute cholangitis was made at the International Consensus Meeting held in Tokyo in 2006. TG07 criteria included: A: Clinical: (1) history of biliary disease; (2) fever and/or chills; (3) jaundice; and (4) abdominal pain (RUQ or epigastric); B: Lab data: (5) evidence of inflammatory response; (6) abnormal liver function tests; C: Imaging findings: (7) biliary dilatation or evidence of an etiology (stone, stricture, stent, *etc.*). Suspected diagnosis: 2 or more items in A. Definite diagnosis: (1) Charcot's triad (2 + 3 + 4); and (2) two or more items in A plus both items in B plus item C.

The sensitivity and specificity of diagnosing acute cholangitis in TG07 were 82.6% and 79.8% respectively. In 2012, TG13, a new Tokyo guideline for the diagnosis of acute cholangitis was published[17]. The criteria included: (1) Systemic inflammation: A-1: Fever (body temperature > 38 °C and/or shaking chills; A-2: Lab data: Evidence of inflammatory response – white blood cell (WBC) count < 4000/cmm or > 10000/cmm, C-reactive protein (CRP) - ⩾ 1 mg/dL; and (2) Cholestasis: B-1: Jaundice-total bilirubin ⩾ 2 mg/dL; B-2: Lab data: Abnormal liver function tests. Alkaline phosphatase (IU) > 1.5 × upper limit of normal; Gamma-glutamyl transpeptidase (IU) > 1.5 × upper limit of normal; Aspartate aminotransferase (IU) > 1.5 × upper limit of normal; Alanine aminotransferase (IU) > 1.5 × upper limit of normal. Imaging: C-1: Biliary dilatation; C-2: Evidence of etiology on imaging (stricture, stone stent, *etc.*).

Suspected diagnosis: One item in A + one item in either B or C. Definite diagnosis: One item in A, one item in B and one item in C. The sensitivity of diagnosing acute cholangitis improved to 91.8% but the specificity remained similar (77.7%) in TG13. The false positive rate of diagnosing acute cholecystitis also decreased to 5.9% in TG13 in comparison to Charcot's triad (11.9%) and TG07 (15.5%).

Physical examination may show high temperature, tachycardia, hypotension, jaundice, right upper quadrant or epigastric tenderness and altered mental status.

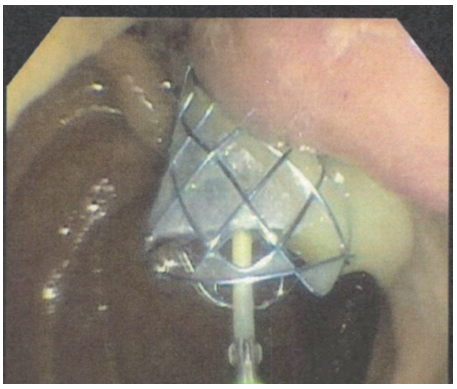

Figure 2 Drainage of pus after biliary stenting during endoscopic retrograde cholangiopancreatography.

Severity of acute cholangitis: two clinical factors determine the severity of acute cholangitis: (1) response to initial medical treatment; and (2) organ dysfunction[1].

Grade Ⅰ is mild acute cholangitis. Patients do not have any organ dysfunction and do not meet the criteria of moderate acute cholangitis. They respond to the initial antibiotic treatment.

Grade Ⅱ is moderate acute cholangitis. Patients do not have any organ dysfunction and do not respond to the initial antibiotic treatment. Any two of the five conditions should be present: (1) leukocytosis (WBC > 12000/cmm) or leukopenia (WBC < 4000/cmm); (2) high temperature (⩾ 39 °C); (3) elderly (age > 75 years); (4) hyperbilirubinemia (total bilirubin ⩾ 5 mg/dL); and (5) hypoalbuminemia (< 0.7 × lower limit of normal).

Grade Ⅲ is severe acute cholangitis. Patients do not respond to initial medical treatment and have organ dysfunction in at least one of the following organs/systems: (1) cardiovascular system: hypotension requiring dopamine infusion ⩾ 5 μg/kg per minute, or any dose of norepinephrine; (2) nervous system: disturbance of consciousness; (3) respiratory system: PaO_2/FiO_2 ratio < 300; (4) renal system: oliguria, serum creatinine > 2 mg/dL; (5) hepatic system: platelet-international normalized ratio (INR) > 1.5; and (6) hematological system: platelet count < 100000/cmm.

Sometimes we make the diagnosis of acute suppurative cholangitis (ASC) when we notice pus extruding from the ampulla of Vater during ERCP (Figures 1 and 2). ASC does not always mean severe acute cholangitis. Sometimes, patients with severe acute cholangitis do not have pus in the bile duct, and sometimes patients with ASC are not that sick[18]. Severe acute cholangitis or toxic cholangitis is present in 5% of all cases of cholangitis[19].

Differential diagnoses of acute cholangitis[20]: (1) acute cholecystitis; (2) cirrhosis of liver; (3) acute hepatitis; (4) liver abscess; (5) septic shock due to any cause; (6) right sided diverticulitis; and (7) righted

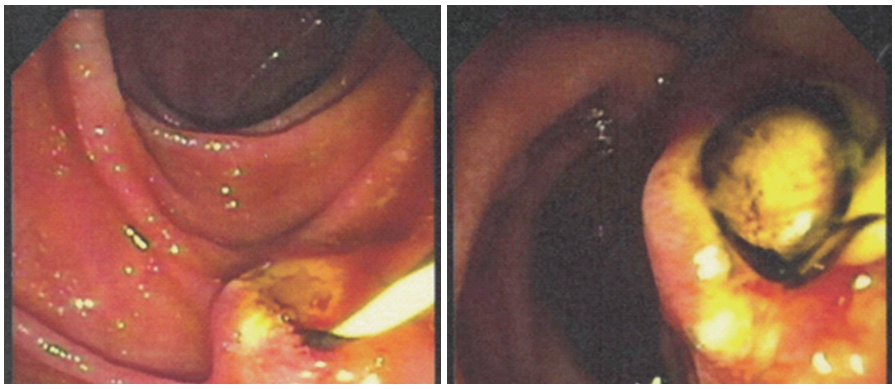

Figure 3 Biliary sphincterotomy followed by stone extraction.

sided pyelonephritis.

Recurrent acute cholangitis can occur when pigment stone is formed in the intrahepatic ducts leading to stricture formation, mainly in the lateral segment of the left lobe or posterior segment of the right lobe[21]. This condition is also called oriental cholangiohepatitis as it occurs almost exclusively in the natives of Southeast Asia. The exact mechanism is not known but related to malnutrition, ascariasis (*Ascaris Lumbricoides*) and clonorchiasis (*Clonorchis sinensis*). Transient portal bacteremia allows entrance of bacteria (*E. coli*, *Klebsiella*, *Pseudomona*, *Proteus*, anaerobes) into the biliary system, initiating a vicious cycle of infection and stone formation[22]. This condition may cause cholangiocarcinoma in 5% of cases.

INVESTIGATIONS

Lab tests should include complete blood count, erythrocyte sedimentation rate or CRP, complete metabolic profile including renal and hepatic function, prothrombin time and INR. Blood culture should be done as early as possible. TG13 guideline also recommends collection of bile sample during the drainage procedure. Bile culture can be positive in 59% to 93% of acute cholangitis cases.

Imaging studies may include ultrasound of the abdomen, regular or helical computed tomography (CT), magnetic resonance cholangiopancreaticography (MRCP) and endoscopic ultrasound (EUS). CT without contrast is more sensitive than abdominal ultrasound in detecting common bile duct stones[23]. Among these, MRCP (82.2% accuracy in detecting choledocholithiasis) and EUS (96.9% accuracy in detecting choledocholithiasis) are the most sensitive imaging modalities, which can detect the level and cause of biliary obstruction[24]. Transabdominal ultrasound is able to detect choledocholithiasis in 30% of cases, and CT in 42% of cases. Although MRCP is being increasingly used in the setting of acute cholangitis, its sensitivity in detecting less than 6 mm stone is low[11].

MANAGEMENT

Patients with cholangitis should be managed at the hospital, as this is considered as an emergent condition. Patients should be resuscitated first. As cholangitis is due to infection and obstruction of the biliary system, we have to treat both aspects. Intravenous fluid and antibiotics should be started as soon as possible. Fresh frozen plasma or vitamin K may be required for correction of coagulopathy. The choice of antibiotics depends on multiple factors, including the patient's renal function, hepatic function, drug allergies, comorbidities, hospital-acquired (multiple or resistant organisms like Pseudomonas, CRE, vancomycin-resistant enterococcus or methicillin-resistant *Staphylococcus aureus*) or community-acquired infection (single agent like *E. coli*, *Klebsiella*, or *Enterococcus*), and also on the severity of cholangitis. The empiric antibiotics should cover both Gram-negative and anaerobic organisms. The initial choice should be piperacillin-tazobactam, ticarcillin-clavulanate, ceftriaxone plus metronidazole or ampicillin-sulbactam. If the patient is sensitive to penicillin, ciprofloxacin plus metronidazole, carbapenems or gentamicin plus metronidazole are good choices[25]. The antibiotics should be further evaluated and adjusted according to the blood culture results. Blood culture is positive in 21% to 71% of cases of acute cholangitis[26]. The dose of the antibiotics should be adjusted according to renal and hepatic functions. Ideally, the antibiotics should be continued for 7 to 10 d[12].

Because of high biliary intraductal pressure, biliary secretion of antibiotics is impaired. So, biliary drainage is the next step. It can be best done by therapeutic ERCP. Depending on the etiology of biliary obstruction, intervention should be done. For example, in case of choledocholithiasis, sphincterotomy and stone extraction (Figures 3-5) should be done with or without transpapillary biliary stent placement. Sometimes, there is an increased risk of bleeding from biliary sphincterotomy if the patient is coagulopathic or on anti-platelet agents. In those cases, biliary stent can be placed temporarily without sphincterotomy. In case of biliary stricture, transpapillary biliary stent placement should give adequate drainage. If there is blockage of the existing stent due to growth of bacterial biofilm and formation of bile sludge, the old stent should be removed and replaced with a new one[27].

Other modalities of biliary drainage include

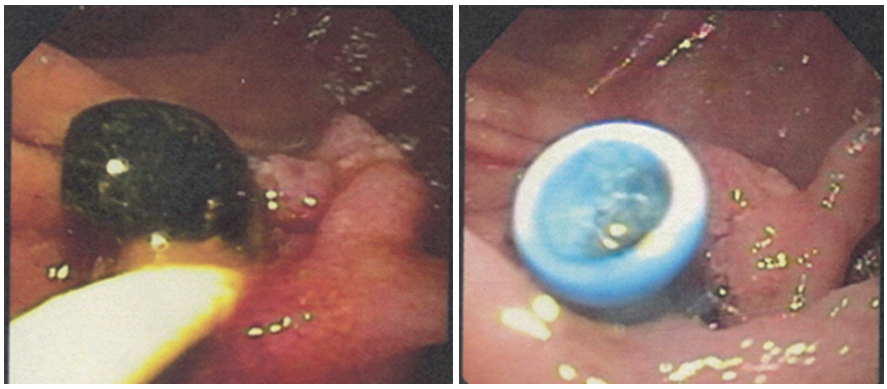

Figure 4 Biliary stone extraction followed by stent placement.

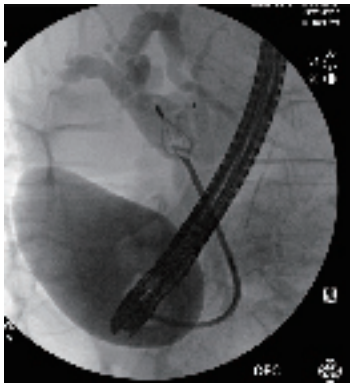

Figure 5 Fluoroscopy showing lithotripsy basket-assisted stone extraction.

Figure 6 Nasobiliary catheter.

endoscopic nasobiliary drainage (ENBD) by nasobiliary catheter (Figure 6), percutaneous transhepatic biliary drainage (PTBD), EUS-guided drainage and open surgical drainage (T-tube drainage after laparotomy).

In clinical practice, ENBD is done much less frequently, as compared to biliary stent placement. ENBD has the advantages that repeat cholangiogram could be done when the location of biliary stricture is not known, thick pus or purulent bile can be drained more effectively, washing can be done if the tube is clogged, biliary aspirate can be cultured and no additional sphincterotomy is required. The disadvantages are that it is uncomfortable to the patient and a confused patient may pull it out[28].

PTBD is generally done in case of failed ERCP or if the patient has multiple comorbidities and is not a good candidate for ERCP. There is no need for intravenous sedation or anesthesia for PTBD. The disadvantages include patient's discomfort, increased length of hospital stay, risks of biliary peritonitis, intraperitoneal hemorrhage and sepsis[29]. It is contraindicated in patients with ascites, coagulopathies and intrahepatic biliary obstructions.

EUS-guided biliary drainage can be performed when ERCP is unsuccessful due to various reasons like ampullary obstruction, gastric outlet obstruction or surgically altered anatomy (Roux-en-Y surgery, gastric bypass, *etc.*), and intrahepatic bile ducts are not dilated[30]. Urgent EUS-guided choledochoduodenostomy with placement of a covered metallic stent is an option in the setting of acute cholangitis[31], mainly in tertiary care centers.

Surgical drainage is reserved when other modalities of biliary drainage are contra-indicated or fail. It is done rarely now-a-days because of high morbidity and mortality of 20% to 60%[32]. To avoid prolonged surgery, choledochotomy with T-tube drainage without choledocholithotomy is recommended[33]. Laparoscopic choledochotomy with stone extraction can be done in case of failed endoscopic extraction of common bile duct stone[34].

In patients with surgically altered anatomy like Roux-en-Y anastomosis or hepaticojejunostomy, balloon enteroscope-assisted ERCP with biliary drainage is done with variable success rate, of 40% to 95%[35].

Timing of biliary drainage: In grade Ⅰ or mild acute cholangitis: Biliary drainage should be done in 24 h to 48 h. In grade Ⅱ or moderate acute cholangitis (*i.e.,* patient has not responded to antibiotics in first 24 h): Early biliary drainage, and in grade Ⅲ or severe acute cholangitis: Urgent biliary drainage should be done. Following endoscopic management of acute cholangitis, laparoscopic cholecystectomy is recommended in patients with gallstone disease[36]. The various techniques of performing laparoscopic cholecystectomy safely have been described over the last few decades[37-39].

Management of recurrent pyogenic cholangitis (Oriental cholangiohepatitis) requires a multidisciplinary team (endoscopist, interventional radiologist and surgeon). Initial treatment includes administration of intravenous fluid and antibiotics, endoscopic treatment with stricture dilation, stone extraction and stent placement for biliary drainage or percutaneous biliary drainage in case of failed ERCP. Segmental hepatic resection should be considered in case of localized disease[40]. Orthotopic liver transplantation has also been reported in case of diffuse disease and end-stage liver disease due to recurrent acute cholangitis[41,42].

PROGNOSIS

The prognosis depends on the timing of biliary drainage, administration of antibiotics and comorbidities of the patient. Early biliary drainage leads to rapid clinical improvement. But, if biliary drainage is delayed, patients can deteriorate quickly and die. The overall mortality acute cholangitis is less than 10% after biliary drainage[43]. In the pre-ERCP era, severe acute cholangitis was associated with a mortality of more than 50%[44]. Emergency surgery for severe acute cholangitis also carries a high mortality, of about 30%[45].

Poor prognostic factors in the setting of acute cholangitis include old age, high fever, leukocytosis, hyperbilirubinemia and hypoalbuminemia[11]. Patients with comorbidities like cirrhosis, malignancy, liver abscess and coagulopathy also carry poor prognosis.

Patients with high pre-biliary drainage serum creatinine is also associated with higher mortality[46]. A recent study also suggested that serum IL-7 level of less than 6.0 and serum procalcitonin level of more than 0.5 was associated with higher mortality[47].

REFERENCES

1 **Wada K**, Takada T, Kawarada Y, Nimura Y, Miura F, Yoshida M, Mayumi T, Strasberg S, Pitt HA, Gadacz TR, Büchler MW, Belghiti J, de Santibanes E, Gouma DJ, Neuhaus H, Dervenis C, Fan ST, Chen MF, Ker CG, Bornman PC, Hilvano SC, Kim SW, Liau KH, Kim MH. Diagnostic criteria and severity assessment of acute cholangitis: Tokyo Guidelines. *J Hepatobiliary Pancreat Surg* 2007; **14**: 52-58 [PMID: 17252297 DOI: 10.1007/s00534-006-1156-7]

2 **Lee SP**, Roberts JR, Kuver R. The changing faces of cholangitis. *F1000Res* 2016; **5**: pii: F1000 [PMID: 27347393 DOI: 10.12688/f1000research.8745.1]

3 **Clough J**, Shah R. Primary Amyloidosis Presenting as Common Bile Duct Obstruction With Cholangitis. *ACG Case Rep J* 2015; **2**: 107-109 [PMID: 26157929 DOI: 10.14309/crj.2015.20]

4 **Uygur-Bayramiçli O**, Ak O, Dabak R, Demirhan G, Ozer S. Taenia saginata a rare cause of acute cholangitis: a case report. *Acta Clin Belg* 2012; **67**: 436-437 [PMID: 23340150 DOI: 10.2143/ACB.67.6.2062709]

5 **Mosler P**. Management of acute cholangitis. *Gastroenterol Hepatol* (N Y) 2011; **7**: 121-123 [PMID: 21475420]

6 **Epstein L**, Hunter JC, Arwady MA, Tsai V, Stein L, Gribogiannis M, Frias M, Guh AY, Laufer AS, Black S, Pacilli M, Moulton-Meissner H, Rasheed JK, Avillan JJ, Kitchel B, Limbago BM, MacCannell D, Lonsway D, Noble-Wang J, Conway J, Conover C, Vernon M, Kallen AJ. New Delhi metallo-β-lactamase-producing carbapenem-resistant Escherichia coli associated with exposure to duodenoscopes. *JAMA* 2014; **312**: 1447-1455 [PMID: 25291580 DOI: 10.1001/jama.2014.12720]

7 **Kimura Y**, Takada T, Strasberg SM, Pitt HA, Gouma DJ, Garden OJ, Büchler MW, Windsor JA, Mayumi T, Yoshida M, Miura F, Higuchi R, Gabata T, Hata J, Gomi H, Dervenis C, Lau WY, Belli G, Kim MH, Hilvano SC, Yamashita Y. TG13 current terminology, etiology, and epidemiology of acute cholangitis and cholecystitis. *J Hepatobiliary Pancreat Sci* 2013; **20**: 8-23 [PMID: 23307004 DOI: 10.1007/s00534-012-0564-0]

8 **Shaffer EA**. Gallstone disease: Epidemiology of gallbladder stone disease. *Best Pract Res Clin Gastroenterol* 2006; **20**: 981-996 [PMID: 17127183 DOI: 10.1016/j.bpg.2006.05.004]

9 What if it's acute cholangitis? *Drug Ther Bull* 2005; **43**: 62-64 [PMID: 16111086 DOI: 10.1136/dtb.2005.43862]

10 **Sung JY**, Costerton JW, Shaffer EA. Defense system in the biliary tract against bacterial infection. *Dig Dis Sci* 1992; **37**: 689-696 [PMID: 1563308 DOI: 10.1007/BF01296423]

11 **Buyukasik K**, Toros AB, Bektas H, Ari A, Deniz MM. Diagnostic and therapeutic value of ERCP in acute cholangitis. *ISRN Gastroenterol* 2013; **2013**: 191729 [PMID: 23997958 DOI: 10.1155/2013/191729]

12 **van den Hazel SJ**, Speelman P, Tytgat GN, Dankert J, van Leeuwen DJ. Role of antibiotics in the treatment and prevention of acute and recurrent cholangitis. *Clin Infect Dis* 1994; **19**: 279-286 [PMID: 7986900 DOI: 10.1093/clinids/19.2.279]

13 **Jain MK**, Jain R. Acute bacterial cholangitis. *Curr Treat Options Gastroenterol* 2006; **9**: 113-121 [PMID: 16539872 DOI: 10.1007/s11938-006-0030-7]

14 **Kinney TP**. Management of ascending cholangitis. *Gastrointest Endosc Clin N Am* 2007; **17**: 289-306, vi [PMID: 17556149 DOI: 10.1016/j.giec.2007.03.006]

15 **Lim JH**. Liver flukes: the malady neglected. *Korean J Radiol* 2011; **12**: 269-279 [PMID: 21603286 DOI: 10.3348/kjr.2011.12.3.269]

16 **Kiriyama S**, Takada T, Strasberg SM, Solomkin JS, Mayumi T, Pitt HA, Gouma DJ, Garden OJ, Büchler MW, Yokoe M, Kimura Y, Tsuyuguchi T, Itoi T, Yoshida M, Miura F, Yamashita Y, Okamoto K, Gabata T, Hata J, Higuchi R, Windsor JA, Bornman PC, Fan ST, Singh H, de Santibanes E, Gomi H, Kusachi S, Murata A, Chen XP, Jagannath P, Lee S, Padbury R, Chen MF, Dervenis C, Chan AC, Supe AN, Liau KH, Kim MH, Kim SW; Tokyo Guidelines Revision Committee. TG13 guidelines for diagnosis and severity grading of acute cholangitis (with videos). *J Hepatobiliary Pancreat Sci* 2013; **20**: 24-34 [PMID: 23307001 DOI: 10.1007/s00534-012-0561-3]

17 **Kiriyama S**, Takada T, Strasberg SM, Solomkin JS, Mayumi T, Pitt HA, Gouma DJ, Garden OJ, Büchler MW, Yokoe M, Kimura Y, Tsuyuguchi T, Itoi T, Yoshida M, Miura F, Yamashita Y, Okamoto K, Gabata T, Hata J, Higuchi R, Windsor JA, Bornman PC, Fan ST, Singh H, de Santibanes E, Gomi H, Kusachi S, Murata A, Chen XP, Jagannath P, Lee S, Padbury R, Chen MF; Tokyo Guidelines Revision Committee. New diagnostic criteria and severity assessment of acute cholangitis in revised Tokyo Guidelines. *J Hepatobiliary Pancreat Sci* 2012; **19**: 548-556 [PMID: 22825491 DOI: 10.1007/s00534-012-0537-3]

18 **Boey JH**, Way LW. Acute cholangitis. *Ann Surg* 1980; **191**: 264-270 [PMID: 7362292 DOI: 10.1097/00000658-198003000-00002]

19 **Lipsett PA**, Pitt HA. Acute cholangitis. *Front Biosci* 2003; **8**: s1229-s1239 [PMID: 12957832]

20 **Scott TM**, Rosh AJ. Acute Cholangitis Differential Diagnoses. Medscape. Updated November 21, 2016. Available from: URL: http://www.medscape.com

21 **Lim JH**. Oriental cholangiohepatitis: pathologic, clinical, and radiologic features. *AJR Am J Roentgenol* 1991; **157**: 1-8 [PMID: 2048504 DOI: 10.2214/ajr.157.1.2048504]

22 **Okuno WT**, Whitman GJ, Chew FS. Recurrent pyogenic cholangiohepatitis. *AJR Am J Roentgenol* 1996; **167**: 484 [PMID: 8686632 DOI: 10.2214/ajr.167.2.8686632]

23 **Gallix BP**, Aufort S, Pierredon MA, Garibaldi F, Bruel JM. [Acute cholangitis: imaging diagnosis and management]. *J Radiol* 2006; **87**: 430-440 [PMID: 16691174 DOI: 10.1016/

S0221-0363(06)74025-2]
24 **de Lédinghen V**, Lecesne R, Raymond JM, Gense V, Amouretti M, Drouillard J, Couzigou P, Silvain C. Diagnosis of choledocholithiasis: EUS or magnetic resonance cholangiography? A prospective controlled study. *Gastrointest Endosc* 1999; **49:** 26-31 [DOI: 10.1016/S0016-5107(99)70441-4]
25 **Qureshi WA**. Approach to the patient who has suspected acute bacterial cholangitis. *Gastroenterol Clin North Am* 2006; **35**: 409-423 [PMID: 16880073 DOI: 10.1016/j.gtc.2006.05.005]
26 **Tanaka A**, Takada T, Kawarada Y, Nimura Y, Yoshida M, Miura F, Hirota M, Wada K, Mayumi T, Gomi H, Solomkin JS, Strasberg SM, Pitt HA, Belghiti J, de Santibanes E, Padbury R, Chen MF, Belli G, Ker CG, Hilvano SC, Fan ST, Liau KH. Antimicrobial therapy for acute cholangitis: Tokyo Guidelines. *J Hepatobiliary Pancreat Surg* 2007; **14**: 59-67 [PMID: 17252298 DOI: 10.1007/s00534-006-1157-6]
27 **Donelli G**, Guaglianone E, Di Rosa R, Fiocca F, Basoli A. Plastic biliary stent occlusion: factors involved and possible preventive approaches. *Clin Med Res* 2007; **5**: 53-60 [PMID: 17456835 DOI: 10.3121/cmr.2007.683]
28 **Nagino M**, Takada T, Kawarada Y, Nimura Y, Yamashita Y, Tsuyuguchi T, Wada K, Mayumi T, Yoshida M, Miura F, Strasberg SM, Pitt HA, Belghiti J, Fan ST, Liau KH, Belli G, Chen XP, Lai EC, Philippi BP, Singh H, Supe A. Methods and timing of biliary drainage for acute cholangitis: Tokyo Guidelines. *J Hepatobiliary Pancreat Surg* 2007; **14**: 68-77 [PMID: 17252299 DOI: 10.1007/s00534-006-1158-5]
29 **Clouse ME**, Evans D, Costello P, Alday M, Edwards SA, McDermott WV Jr. Percutaneous transhepatic biliary drainage. Complications due to multiple duct obstructions. *Ann Surg* 1983; **198**: 25-29 [PMID: 6859989]
30 **Karaliotas C**, Sgourakis G, Goumas C, Papaioannou N, Lilis C, Leandros E. Laparoscopic common bile duct exploration after failed endoscopic stone extraction. *Surg Endosc* 2008; **22**: 1826-1831 [PMID: 18071799 DOI: 10.1007/s00464-007-9708-8]
31 **Artifon EL**, Ferreira FC, Sakai P. Endoscopic ultrasound-guided biliary drainage. *Korean J Radiol* 2012; **13** Suppl 1: S74-S82 [PMID: 22563291 DOI: 10.3348/kjr.2012.13.S1.S74]
32 **Minaga K**, Kitano M, Imai H, Yamao K, Kamata K, Miyata T, Omoto S, Kadosaka K, Yoshikawa T, Kudo M. Urgent endoscopic ultrasound-guided choledochoduodenostomy for acute obstructive suppurative cholangitis-induced sepsis. *World J Gastroenterol* 2016; **22**: 4264-4269 [PMID: 27122677 DOI: 10.3748/wjg.v22.i16.4264]
33 **Liu CL,** Fan ST. Acute cholangitis. In: Surgical Treatment: Evidence-Based and Problem-Oriented. Holzheimer RG, Mannick JA, editors. Munich: Zuckschwerdt, 2001
34 **Tsuyuguchi T**, Takada T, Kawarada Y, Nimura Y, Wada K, Nagino M, Mayumi T, Yoshida M, Miura F, Tanaka A, Yamashita Y, Hirota M, Hirata K, Yasuda H, Kimura Y, Strasberg S, Pitt H, Büchler MW, Neuhaus H, Belghiti J, de Santibanes E, Fan ST, Liau KH, Sachakul V. Techniques of biliary drainage for acute cholangitis: Tokyo Guidelines. *J Hepatobiliary Pancreat Surg* 2007; **14**: 35-45 [PMID: 17252295 DOI: 10.1007/s00534-006-1154-9]
35 **Itoi T**, Tsuyuguchi T, Takada T, Strasberg SM, Pitt HA, Kim MH, Belli G, Mayumi T, Yoshida M, Miura F, Büchler MW, Gouma DJ, Garden OJ, Jagannath P, Gomi H, Kimura Y, Higuchi R; Tokyo Guideline Revision Committee. TG13 indications and techniques for biliary drainage in acute cholangitis (with videos). *J Hepatobiliary Pancreat Sci* 2013; **20**: 71-80 [PMID: 23307008 DOI: 10.1007/s00534-012-0569-8]
36 **Poon RT**, Liu CL, Lo CM, Lam CM, Yuen WK, Yeung C, Fan ST, Wong J. Management of gallstone cholangitis in the era of laparoscopic cholecystectomy. *Arch Surg* 2001; **136**: 11-16 [PMID: 11146767 DOI: 10.1001/archsurg.136.1.11]
37 **Strasberg SM**, Hertl M, Soper NJ. An analysis of the problem of biliary injury during laparoscopic cholecystectomy. *J Am Coll Surg* 1995; **180**: 101-125 [PMID: 8000648]
38 **Callery MP**. Avoiding biliary injury during laparoscopic cholecystectomy: technical considerations. *Surg Endosc* 2006; **20**: 1654-1658 [PMID: 17063288 DOI: 10.1007/s00464-006-0488-3]
39 **Hori T**, Oike F, Furuyama H, Machimoto T, Kadokawa Y, Hata T, Kato S, Yasukawa D, Aisu Y, Sasaki M, Kimura Y, Takamatsu Y, Naito M, Nakauchi M, Tanaka T, Gunji D, Nakamura K, Sato K, Mizuno M, Iida T, Yagi S, Uemoto S, Yoshimura T. Protocol for laparoscopic cholecystectomy: Is it rocket science? *World J Gastroenterol* 2016; **22**: 10287-10303 [PMID: 28058010 DOI: 10.3748/wjg.v22.i47.10287]
40 **Cosenza CA**, Durazo F, Stain SC, Jabbour N, Selby RR. Current management of recurrent pyogenic cholangitis. *Am Surg* 1999; **65**: 939-943 [PMID: 10515539]
41 **Jeyarajah DR**. Recurrent Pyogenic Cholangitis. *Curr Treat Options Gastroenterol* 2004; **7**: 91-98 [PMID: 15010022 DOI: 10.1007/s11938-004-0029-x]
42 **Strong RW**, Chew SP, Wall DR, Fawcett J, Lynch SV. Liver transplantation for hepatolithiasis. *Asian J Surg* 2002; **25**: 180-183 [PMID: 12376243 DOI: 10.1016/S1015-9584(09)60170-6]
43 **Zhang WZ**, Chen YS, Wang JW, Chen XR. Early diagnosis and treatment of severe acute cholangitis. *World J Gastroenterol* 2002; **8**: 150-152 [PMID: 11833092 DOI: 10.3748/wjg.v8.i1.150]
44 **Andrew DJ**, Johnson SE. Acute suppurative cholangitis, a medical and surgical emergency. A review of ten years experience emphasizing early recognition. *Am J Gastroenterol* 1970; **54**: 141-154 [PMID: 5458220]
45 **Lai EC**, Tam PC, Paterson IA, Ng MM, Fan ST, Choi TK, Wong J. Emergency surgery for severe acute cholangitis. The high-risk patients. *Ann Surg* 1990; **211**: 55-59 [PMID: 2294844 DOI: 10.1097/00000658-199001000-00009]
46 **Tai DI**, Shen FH, Liaw YF. Abnormal pre-drainage serum creatinine as a prognostic indicator in acute cholangitis. *Hepatogastroenterology* 1992; **39**: 47-50 [PMID: 1568708]
47 **Suwa Y**, Matsuyama R, Goto K, Kadokura T, Sato M, Mori R, Kumamoto T, Taguri M, Miyasho T, Endo I. IL-7 and procalcitonin are useful biomarkers in the comprehensive evaluation of the severity of acute cholangitis. *J Hepatobiliary Pancreat Sci* 2017; **24**: 81-88 [PMID: 28002647 DOI: 10.1002/jhbp.420]

P- Reviewer: Hori H, Sergi CM, Tamori A **S- Editor**: Qi Y
L- Editor: Filipodia **E- Editor**: Song XX

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: http://www.f6publishing.com

World J Gastrointest Pathophysiol 2018 February 15; 9(1): 8-17

DOI: 10.4291/wjgp.v9.i1.8

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Emergency resection surgery for colorectal cancer: Patterns of recurrent disease and survival

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Author contributions: Subar D and Curran F designed the study interpreted findings and prepared manuscript; Littlechild J, Junejo M, Simons AM collected the data, interpreted findings and prepared manuscript.

Institutional review board statement: The study proposal was reviewed, approved and registered by the Audit and Research Department of the Central Manchester Foundation Trust NHS hospital.

Informed consent statement: Patients were not required to give informed consent to the study. Anonymised data was collected and evaluated in a retrospective study following a significant period after primary intervention.

Conflict-of-interest statement: The authors have no conflict of interest.

Data sharing statement: None.

Manuscript source: Unsolicited manuscript

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Received: March 23, 2017
Peer-review started: March 24, 2017
First decision: May 4, 2017
Revised: November 25, 2017
Accepted: December 4, 2017
Article in press: December 5, 2017
Published online: February 15, 2018

Abstract

AIM

To evaluate prognostic pathological factors associated with early metachronous disease and adverse long-term survival in these patients.

METHODS

Clinical and histological features were analysed retrospectively over an eight-year period for prognostic impact on recurrent disease and overall survival in patients undergoing curative resection of a primary colorectal cancer.

RESULTS

A total of 266 patients underwent curative surgery during the study period. The median age of the study cohort was 68 year (range 26 to 91) with a follow-up of 7.9 years (range 4.6 to 12.6). Resection was undertaken electively in 225 (84.6%) patients and emergency resection in 35 (13.2%). Data on timing of surgery was missing in 6 patients. Recurrence was noted in 67 (25.2%) during the study period and was predominantly early within 3 years (82.1%) and involved hepatic metastasis in 73.1%. Emergency resection (OR = 3.60, P = 0.001), T4 stage (OR = 4.33, P < 0.001) and lymphovascular invasion (LVI)

(OR = 2.37, *P* = 0.032) were associated with higher risk of recurrent disease. Emergency resection, T4 disease and a high lymph node ratio (LNR) were strong independent predictors of adverse long-term survival.

CONCLUSION

Emergency surgery is associated with adverse disease free and long-term survival. T4 disease, LVI and LNR provide strong independent predictive value of long-term outcome and can inform surveillance strategies to improve outcomes.

Key words: Emergency resection; Colorectal cancer; Metachronous disease; Lymph node ratio; Survival

Core tip: Despite increasing uptake of national bowel cancer screening programme in the United Kingdom, majority of patients with colorectal cancer are diagnosed following the urgent 2-wk referral or present as an emergency (53%). Emergency resection surgery for colorectal cancer is associated with a high post-operative morbidity and mortality and adverse long-term survival compared to elective surgery. Although immediate survival may be affected by factors associated with provision of emergency surgery and critical care, long-term disease recurrence and survival is dictated by presence of adverse clinical and histological factors which can guide post-operative surveillance for recurrent disease.

Littlechild J, Junejo M, Simons AM, Curran F, Subar D. Emergency resection surgery for colorectal cancer: Patterns of recurrent disease and survival. *World J Gastrointest Pathophysiol* 2018; 9(1): 8-17 Available from: URL: http://www.wjgnet.com/2150-5330/full/v9/i1/8.htm DOI: http://dx.doi.org/10.4291/wjgp.v9.i1.8

INTRODUCTION

Colorectal cancer is the fourth most common cancer in the United Kingdom with over 40000 cases diagnosed each year[1]. At presentation, synchronous hepatic metastases are present in 20%-25% of patients with metachronous hepatic metastases developing in 40%-50%[2,3]. Up to 80% of patients who develop metachronous disease do so within the first 3 years[4,5]. About 10% of patients who have resection of the primary tumour with curative intent will develop metachronous lung metastases[6] with local recurrence having been reported to occur in 4%-10% of patients[7,8]. Recent decades have seen a significant improvement in early post-operative survival in patients undergoing lung or liver resection surgery for colorectal metastasis[9,10]. Intensive post-operative surveillance has shown to improve 5-year survival with recognition of early, asymptomatic recurrent disease[11]. Five-year survival has further improved with the advent of effective chemotherapy, especially for patients with resectable liver disease, seeing a rise from 35% to 50%[10,12]. Three meta-analyses[13-15] have shown improved, albeit modest, survival with aggressive surveillance, which has been proven to be within the National Health Service's threshold of cost acceptability[16].

Despite the widespread use of screening programs for detection of colorectal cancer, a large number of cases in England are diagnosed in the acute or urgent setting either as an emergency presentation (26%) or following an urgent 2-wk referral (27%)[17]. Compared to elective resection, emergency surgery is associated with adverse postoperative outcomes (post-operative mortality 4.6% *vs* 16%), disease-free and overall long-term survival[18-20]. This may represent a multifactorial basis due to altered physiology, immunosuppression, advanced disease and aggressive tumour biology[21,22].

Surveillance after resection of the colorectal primary in the form of colonoscopy and computed tomography (CT) imaging with or without adjuncts such as positron emission tomography (PET/PET-CT) is generally considered the standard of care[4]. There is wide variation in follow-up protocols in randomised trials with no clear consensus about follow-up intensity[23]. The Association of Coloproctologists of Great Britain and Ireland (ACPGBI) recommends a CT scan within the first 2 years after resection of the primary tumour to detect metastases as part of the follow up of these patients[24]. Conversely, the American Society of Clinical Oncology (ASCO) has suggested an annual abdominal/chest CT scan for three years with more frequent scans for higher risk patients (defined as stage III or stage II with multiple high-risk features)[4]. The American National Comprehensive Cancer Network (NCCN) recommends an abdominal/pelvic and chest CT scan annually for up to five years[25]. Furthermore, it is clear that not all patients with colorectal cancer will benefit from intensive surveillance, incurring unnecessary costs. Identifying patients at high risk of developing early recurrence (within the first three years) will help to determine who would benefit from aggressive surveillance.

The aim of this study was to investigate the prognostic effects of histological factors on patterns of recurrent disease and survival in patients undergoing emergency resection surgery for colorectal cancer.

MATERIALS AND METHODS

A retrospective audit of patients undergoing surgery for colorectal cancer was carried out after institutional approval. Patients undergoing consecutive curative resection for histologically proven, primary colorectal adenocarcinoma in the absence of synchronous metastatic disease on presentation were included the study covering an eight-year period from January 2001 to December 2008. The study population was identified

using procedure codes from the hospital database.

Demographic data on age, sex and mode of presentation was recorded. In the emergency surgery setting the presence of bowel obstruction or perforation was recorded. Histological data was retrieved for site of tumour, TNM staging was based on postoperative histological findings along with degree of tumour differentiation, lymphovascular invasion (LVI), perineural invasion (PNI), resection margin status (R). Lymph node ratio (LNR) was calculated as the ratio of disease positive nodes to the total number of lymph nodes retrieved. Clinic letters and correspondences within the computerised hospital database were accessed to gain information on presentation, mode of surgery and neo-adjuvant and adjuvant therapies. Treatment decisions regarding neo-adjuvant and adjuvant therapies were carried out in multi-disciplinary team meetings based on patient fitness, symptoms, synchronous disease and post-operative recovery. Generally, patients with rectal tumour received neo-adjuvant therapy if the tumour was > T3b or in presence of nodal disease on cross-sectional imaging.

Surveillance following curative colorectal cancer resection incorporated 6 monthly clinical assessments for 3 years followed by annual reviews for a total of 5 years for patients without recurrent disease. This assessment was supplemented by 6 monthly CEA levels (Carcinoembroyic Antigen), CT of chest, abdomen and pelvis within 2 years of surgery and 5-yearly surveillance colonoscopy (following completion colonoscopy within a year where indicated) until the age of 75 years (or longer if life expectancy deemed longer than 10 years at this stage). Detection of new symptoms, increased CEA levels, and abnormal CT or colonoscopy findings warranted multidisciplinary team review during follow-up. Patients developing an extra-colonic primary malignancy during the course of follow-up were excluded from the study to minimise bias. Survival data was obtained as all-cause mortality using the Demographics Batch Service (DBS) to access the national electronic database of the United Kingdom NHS.

Primary outcome was development of early re-currence disease within three years of curative resection. This was subdivided into liver, lung and local recurrence. Secondary outcomes were overall recurrence, three-year and overall survival.

Statistical analysis was performed using SPSS® version 20.0 (IBM, New York, NY, United States) using non-parametric tests (χ^2 test and logistic regression). Continuous data in the text are reported as median (range), unless stated otherwise. $P < 0.050$ was regarded as significant. Variables with $P > 0.100$ on univariate analysis were excluded from multiple regression predictive model analyses. Models with multiple variables were assessed for interactions. Receiver operating characteristic (ROC) curves were plotted for continuous variables to estimate threshold values that differentiated groups. Survival analysis was carried out using the Kaplan-Meier method. Differences in survival curves were assessed using the log rank method.

RESULTS

The database identified 266 patients meeting the inclusion criteria during the study period. Median period of follow-up was 7.9 years (4.6 to 12.6 years). The median age at the time of surgery was 68 years (26 to 91 years) with a male to female ratio of 1.5:1.1. Surgery was undertaken as an elective procedure in 225 patients (84.6%) and as an emergency in 35 (13.2%) patients and remained unclear for 6 (2.2%) patients. The indication for emergency surgery was not identified in 3 patients. Bowel obstruction was the predominant indication for emergency surgery, undertaken in 28 patients compared to 9 patients with a diagnosis of perforation on presentation.

Of the total 266 patients, 151 were diagnosed with tumours situated in the colon and 115 within the rectum. Data on neo-adjuvant therapy was missing in 12 patients (7 colonic and 5 rectal cancers). None of the remaining patients with colon cancer received neo-adjuvant therapy before surgery. Of 115 patients with rectal cancer, 39 (33.91%) received pre-operative radiotherapy, 10 (8.69%) received pre-operative chemo-radiotherapy and 5 (4.31%) had pre-operative chemotherapy alone. Postoperatively, amongst patients with tumour sited in the colon, only 53 (35.10%) received adjuvant therapies (data missing in 6 patients). Amongst patients with rectal cancer, 54 (46.96%) received neo-adjuvant therapy and 14 of these patients proceeded to have further adjuvant therapies post-operatively. Overall, 30 (26.09%) patients with rectal cancer had adjuvant therapies post-operatively (missing data in 3).

The site and histological features of the primary colorectal tumour in all 266 patients are shown in Table 1. TNM staging is presented from postoperative histological assessment. Pre-operative radiological changes in staging before and after neo-adjuvant therapy were not collected. Although T stage was not recorded for 18 patients positive residual disease was identified in 16 of these patients. Lymph node status was missing in 17 patients.

Emergency surgery for colorectal cancer

Complete data was available from 35 patients undergoing emergency colorectal cancer resection. Detailed characteristics of patients undergoing emergency and elective colorectal resection for cancer are shown in Table 2. No significant differences were noted between the two groups in age, sex, tumour grade, nodal disease, LNR, LVI, PNI or resection margin status on univariate regression analysis. Right colon was more commonly the site of primary cancer in the emergency group and associated with higher rate of obstruction. More patients undergoing emergency

Table 1 Site of primary tumour and histological features in 266 curative resections for colorectal cancer *n* (%)

Tumour site	
Rectum	115 (43.23)
Recto-sigmoid junction	2 (0.75)
Sigmoid	64 (24.06)
Descending/splenic flexure	7 (2.63)
Transverse colon	14 (5.26)
Ascending/hepatic flexure	15 (5.64)
Caecum	49 (18.42)
Tumour size[1]	
T1	8 (3.00)
T2	54 (20.30)
T3	121 (45.49)
T4	66 (24.81)
Missing	18 (6.77)
Nodal status and invasion[1]	
N0	154 (57.89)
N1	65 (24.43)
N2	30 (11.28)
LVI (data missing in 15)	60 (22.56)
PNI (data missing in 15)	14 (5.26)
Resection margin (data missing in 16)	
R0	229 (86.09)
R1	14 (5.26)
R2	7 (2.63)
Tumour differentiation (data missing in 17)	
Well	22 (8.27)
Moderately	176 (66.17)
Poor	51 (19.17)

[1]Values given in parenthesis are percentages. Disease staging was based on postoperative histology findings and stage was not compared with preoperative radiological staging or post neoadjuvant therapy. T: Tumour size; N: Nodal status; LVI: Lymphovascular invasion; PNI: Perineural invasion; R: Resection margin.

Table 2 Patient characteristics for emergency and elective colorectal cancer resection

Variables	Emergency surgery	Elective surgery	P^2
n	35	225	
Median age in years	70 (41-85)	68 (26-91)	NS
Sex (males/females)	20/15	128/97	NS
Site of CRC			-
Right colon	17 (48.6%)	56 (24.8%)	< 0.001
Left colon	15 (42.9%)	58 (25.8%)	NS
Rectum	3 (8.6%)	111 (49.3%)	< 0.001
Obstruction	26 (74.3%)	2[1] (0.9%)	< 0.001
Right colon	15 (42.9%)	1 (0.4%)	-
Left colon	10 (28.6%)	0	-
Rectum	1 (2.9%)	1 (0.4%)	-
Perforation	9 (25.7%)	0	< 0.001
Right colon	2 (5.7%)	0	-
Left colon	5 (14.3%)	0	-
Rectum	2 (5.7%)	0	-
Neoadjuvant therapy	1 (2.9%)	53 (23.6%)	0.021
Adjuvant therapy	16 (45.7%)	65 (28.8)	0.047
Poorly differentiated	5 (14.3%)	44 (19.6%)	NS
T4 disease	18 (51.4%)	46 (20.4%)	< 0.001
Positive resection margin	2 (5.7%)	19 (8.4%)	NS
N2 disease	15 (42.9%)	78 (34.7%)	NS
Liver metastasis	16 (42.1%)	30 (13.3%)	< 0.001
Early (< 3 yr)	14 (40.0%)	26 (11.6%)	-
Lung metastasis	3 (8.6%)	10 (4.4%)	NS
Early (< 3 yr)	3 (8.6%)	8 (3.6%)	-
Local metastasis	1 (2.9%)	9 (4.0%)	NS
Early (< 3 yr)	1 (2.9%)	6 (2.7%)	-
Overall recurrence	17 (48.6%)	47 (21.3%)	0.001
Median recurrence in days	280 (112-1155)	420 (70-1855)	NS
Median DFS in days	1120 (105-3892)	2302 (704522)	0.004
Median survival in days	1913 (105-3892)	2359 (194-4522)	0.040

[1]Subacute obstructed were managed as a planned resection. Six patients were excluded for missing data from the study cohort of 266 patients; [2]Significance at $P < 0.05$. CRC: Colorectal cancer; DFS: Disease free survival; NS: Not significant.

surgery had T4 disease stage on univariate analysis (OR = 4.33, 95%CI: 2.03-9.25, $P < 0.001$) and a higher proportion received adjuvant therapies post-resection compared to elective resection (45.7% *vs* 28.8%, OR = 2.10, 95%CI: 1.01-4.37, $P = 0.047$). Multivariate modelling was not carried out as no other histological parameter reached significance of $P < 0.100$.

Emergency resection was also positively associated with increased risk of recurrent disease on univariate analysis (48.57% *vs* 20.89%, OR = 3.60, 95%CI: 1.69-7.64, $P = 0.001$) with liver being the commonest site of early recurrent disease (40.0% *vs* 11.6%, OR = 5.78, 95%CI: 2.66-12.55, $P < 0.001$). A subset analysis demonstrated a greater proportion of patients undergoing emergency resection received adjuvant therapies compared to elective resection (45.7% *vs* 28.9%). In the emergency surgery group, patients with T4 disease were likely to received adjuvant therapies ($P = 0.004$). These patients receiving adjuvant therapies demonstrated an increased trend towards risk of liver metastasis but it failed to reach significance ($P = 0.089$). No differences were seen in recurrence rates for lung and local disease in the two groups. Although, median period to recurrence in days was shorter in the emergency resection group, this did not reach significance (OR = 0.99, 95%CI: 0.99-1.00, $P = 0.181$).

Patients undergoing emergency resection also displayed adverse disease free survival (DFS) and overall survival compared to elective resections. Median DFS and overall survival were poorer in the emergency resection at 1155 days (Log rank $P < 0.001$, Figure 1) and 1931 d (Log rank $P = 0.027$, Figure 2) respectively.

Patterns of recurrent disease and survival

Recurrent disease was observed in 67 out of 264 patients with available data (25.38%) during the median follow-up period of 7.9 years (range 4.6 to 12.6 years). Amongst patients with recurrence, liver was the commonest site in 49 out of 67 (73.13%) patients with isolated liver disease in 41 patients. This was followed by lung disease in 14 (20.90%) patients with isolated disease in 4. Local recurrence was seen in 10 (14.93%) patients and 6 of whom had isolated local disease. Ten patients developed recurrence at multiple sites; 7 (10.45%) with concomitant liver and lung and 3 (4.58%) patients had lung metastasis with local recurrence.

Recurrence was predominantly early (< 3 years) affecting 55 patients (Table 3). The liver remained the commonest site of early recurrent disease with 35 out

Table 3 Site of early (< 3 yr) and overall recurrent disease during follow-up

Site of recurrence	Early recurrence (*n* = 55)	Colonic cancer (*n* = 151)	Rectal cancer (*n* = 115)	*P*[1]
Liver	41 (74.55%)	25	16	0.554
Lung	11 (20.00%)	6	5	0.879
Local	7 (12.73%)	4	3	0.984
> 1 site	8 (14.55%)	4	4	0.695

[1]Significance at $P < 0.05$, colonic cancer *vs* rectal cancer. Number in parenthesis is percentage unless stated otherwise.

Table 4 Prognostic factors for early liver recurrence

Early liver recurrence	B	SE		OR	95%CI
T4	0.453	0.440	0.303	1.574	0.664-3.729
N2	0.706	0.540	0.191	2.025	0.703-5.831
LVI	0.974	0.456	0.033	2.648	1.084-6.468
PNI	0.735	0.700	0.294	2.085	0.529-8.214
R1	0.954	0.726	0.189	2.597	0.626-10.777
Obstruction	1.385	0.514	0.007	3.995	1.457-10.949
Perforation	1.958	0.781	0.012	7.086	1.533-32.749

B: Regression coefficient; SE: Standard error; OR: Odds ratio; T: Tumour size; N: Nodal status; LVI: Lymphovascular invasion; PNI: Perineural invasion; R: Resection margin.

of 41 patients presenting with isolated liver disease. Early multi-site disease was seen in a small proportion of patients with liver disease (4 patients with liver and lung disease, 2 with liver and lung and local disease). In contrast, isolated lung disease was noted in only 3 out of 11 patients with early disease (4 patients had lung and liver disease, 2 had lung disease with local recurrence, 2 had lung, liver and local recurrence). Local recurrence was seen in 7 patients and was isolated in 3.

Recurrence between 3 to 5 years following curative resection represented 14.92% (10 patients) of overall recurrent disease. The liver remained the predominant site with 5 patients demonstrating isolated liver disease and 1 with liver and lung metastasis. Two patients presented with lung disease (1 isolated and 1 combined lung and liver disease) and 3 patients presented with isolated late local recurrence. Beyond the 5-year follow-up period, 1 patient developed isolated liver disease and 1 had liver and lung metastasis. No local recurrence was noted beyond 5-year follow-up.

Age, sex, tumour location, tumour differentiation and neo-adjuvant therapy did not show correlation with early all-site recurrent disease. Significant histological markers of early recurrence on univariate analyses included T4 stage (OR = 3.61, 95%CI: 1.88-6.96, $P < 0.001$), N2 nodal status (OR = 4.18, 95%CI: 1.89-9.24, $P < 0.001$), LVI (OR = 3.40, 95%CI: 1.79-6.46, $P < 0.001$), PNI (OR = 4.54, 95%CI: 1.51-13.60, $P < 0.007$) and R1 resection margin (OR = 4.51, 95%CI: 1.50-13.53, $P = 0.007$). LNR provided a strong correlation for early all-site recurrence on univariate analyses (OR = 25.55, 95%CI: 4.52-144.32, $P < 0.001$) with an optimal cut-off at 0.015 (52.7% sensitivity and 68.4% specificity). In a multivariate predictive model for early all-site recurrence, emergency surgery with perforation was the strongest predictor amongst factors including T4 stage and LVI.

For early liver disease, no correlation was noted with age, sex, site of tumour or tumour differentiation on univariate analysis. Emergency surgery was associated with a higher risk of recurrent disease in the liver (OR = 5.13, 95%CI: 2.33-11.30, $P < 0.001$), with an adverse outcome in the presence of perforation than obstruction alone (OR 4.78 *vs* 3.74). Histological factors demonstrating significance on univariate analysis included T4 stage (OR = 2.76, 95%CI: 1.32-5.76, $P = 0.007$), N2 stage (OR = 3.32, 95%CI: 1.42-7.76, $P = 0.006$), LVI (OR = 3.02, 95%CI: 1.49-6.10, $P = 0.002$), PNI (OR = 3.71, 95%CI: 1.17-11.80, $P = 0.026$) and R1 resection margin (OR = 3.70, 95%CI: 1.16-11.74, $P = 0.027$). LNR (OR = 11.69, 95%CI: 1.94-70.24, $P = 0.007$) provided AUC of 0.61 (95%CI: 0.51-0.71, $P = 0.023$) with the optimal cut-off at 0.015 (53.7% sensitivity and 67.3% specificity). A multivariate predictive model of presentation and histological features showed that perforation at the time of surgery was the strongest independent predictor of early liver recurrence amongst other markers of predictive value (Table 4).

No independent predictors were identified for early lung recurrence. Early local recurrence represented a very small number of patients (7) for detailed analyses.

Long-term survival data was available from 262 patients with a median survival of 9.9 years. One-, three- and five-year survival in the study cohort was 96%, 82% and 72% respectively. Emergency surgery was associated with poorer survival (log rank $P = 0.027$) with 1, 3 and 5 year survival at 90%, 65% and 50% respectively (Figure 2). Multivariate Cox regression demonstrated T4 status to be a stronger predictor

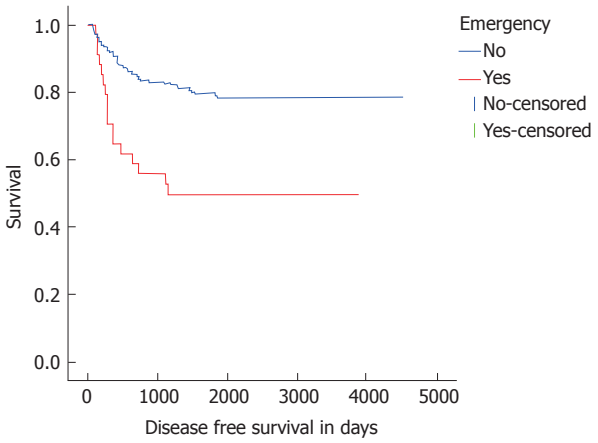

Figure 1 Median disease free survival and overall survival were poorer in the emergency resection at 1155 d (Log rank *P* < 0.001).

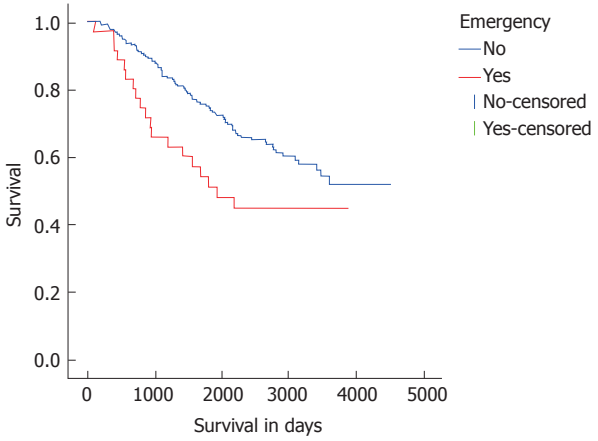

Figure 2 Median disease free survival and overall survival were poorer in the emergency resection at 1931 d (Log rank *P* = 0.027).

of adverse outcome than emergency surgery (HR 1.96, 95%CI: 1.27 to 3.02, *P* = 0.003). No significant difference in long-term survival was noted in patients with colonic or rectal disease (log rank *P* = 0.217).

Amongst histological features, T4 disease (HR = 1.65, 95%CI: 1.05-2.60, *P* = 0.031), PNI (HR = 3.20, 95%CI: 1.72-5.97, *P* < 0.001) and LNR were independent predictors of adverse outcome in a multivariate Cox regression model. LNR was associated with the greatest risk of early death (HR = 11.87, 95%CI: 3.98-35.36, *P* < 0.001).

Median survival with early recurrence was 2.62 years (95%CI: 1.92-3.31). In the presence of early liver recurrence, median survival was 2.78 years (95%CI: 2.27-3.39); early lung disease was 2.17 years (95%CI: 1.82-2.53) and early local recurrence was 1.80 years (95%CI: 1.27-2.33). For overall recurrence, isolated liver or lung only metastatic disease showed a better survival pattern than multi-site or local recurrent disease (Figure 3). Median survival in isolated liver disease was 2.87 years (95%CI: 2.39-3.35), which was not significantly different compared to 3.98 years (95%CI: 1.12-6.85) for isolated lung recurrence. Multi-site disease and local disease were associated with significantly adverse survival compared to isolated liver disease.

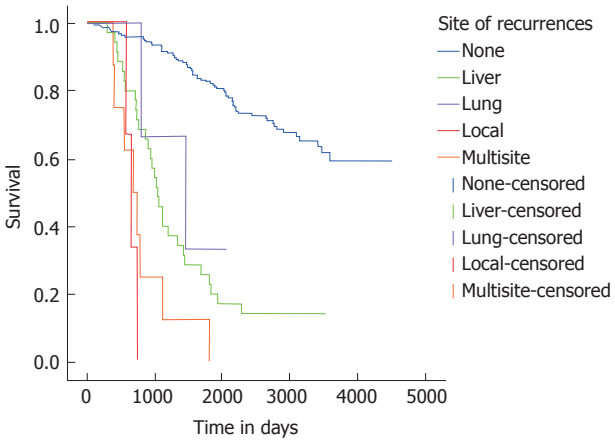

Figure 3 For overall recurrence, isolated liver or lung only metastatic disease showed a better survival pattern than multi-site or local recurrent disease.

DISCUSSION

Emergency resection for colorectal cancer presents a high risk group with increased risk of early recurrence and adverse long-term survival. Right colonic tumours and T4 stage are prognostic predictors for emergency colorectal cancer resection. T4 stage, LVI and emergency resection also offer a strong prognostic prediction for development of recurrent disease identifying high-risk patients for aggressive surveillance or adjuvant therapies.

Emergency colorectal resection constituted 13.2% of patients undergoing curative surgery and did not constitute all emergency presentations with symptomatic colorectal cancer. This is smaller than the reported national average of up to 30%[17,26] and may be the result of patient selection practices and the emerging role of self-expanding metal stent for acute malignant large bowel obstruction. Emergency surgery in our cohort was associated with poorer early and long-term survival. Hogan *et al*[20] concluded similarly that emergent surgery was associated with higher rates of local recurrence and poorer disease-free survival. Patients presenting in an emergency setting with advanced disease may have a distinct, aggressive tumour with unfavourable biology[22] and therefore these patients may be more prone to early recurrence. Furthermore, in the emergency setting patients have altered physiology and are immunosuppressed so that tumour dissemination in these circumstances occurs more easily[21]. Correlation between the increased morbidity of emergency surgery and timely or successful progression to adjuvant therapies was not specifically evaluated. The use of self-expanding colonic stents offers to address the associated high morbidity and mortality with emergency surgery[27,28].

Lymph node involvement, along with depth of bowel

wall invasion, has represented an important prognostic indicator for metachronous metastasis[29]. This is in broad correlation with our study. Nodal status was noted to be an independent predictor in early all-site recurrence, but no independent predictive value was noted for early liver and lung recurrent disease. In the setting of emergency surgery, where adequate lymph node yield may be compromised, LNR may prove to be a more useful prognostic tool since the patient's physiology might not tolerate prolonged surgery in order to attain a good lymph node yield.

Keum *et al*[30] concluded that high risk factors for recurrence include rectal cancer, T2 stage and an infiltrative growth pattern. However, this study only looked at patients who underwent resection for stage I colorectal cancer which may explain their finding of T2 stage compared with ours of T4 stage. It has been stated previously that rectal tumours and younger age at presentation have a higher recurrence risk[31]. However, this was not the case in our study for reasons that are not clear to us.

LVI was also found to be an independent predictor of early all-site recurrence in a similar study by Huh *et al*[32] however they classified early recurrence as less than 1 year after operation. Lim *et al*[33] found that after attempted curative resection, patients with LVI-positive tumours had a higher rate of all-site recurrence than those without LVI. This is in correlation with our study.

Up to 50% of patients develop hepatic metastases within the first three years after curative resection[3]. In our study, overall recurrence occurred in 25% of the patient population, with 82% of the recurrence diagnosed within three years after resection. This is commensurate with the reported literature[4,34]; Meyerhardt *et al*[4] reports 80% of recurrences occur within the first three years. The liver was by far the most common site for early recurrence with 74% of cases occurring there. This is due to haematogenous spread *via* the portal venous system[35]. Pietra *et al*[36] also reported that 65% of recurrence occurred in the liver, in keeping with our observation.

Median survival of patients with early liver recurrence was 2.78 years. This compares favourably with patients presenting with early local recurrence who had a median survival of 1.8 years. This improved outcome may be a reflection of a more aggressive approach of isolated liver disease with the advent of improved liver parenchyma sparing techniques and advances in surgical techniques and perioperative care. The 5-year survival rate for patients that undergo curative metachronous resection of four or less hepatic lesions is 24%-58%[37-40]. A 5-year survival of 24% has also been reported for curative metachronous resection of > 8 hepatic lesions[41]. Detection of metachronous disease as early as possible is imperative in limiting the extent of resection and improving survival. Renehan *et al*[15] highlighted the value of intensive follow-up by demonstrating a high detection of early metachronous disease by aid of CEA and computed tomography. This has delivered an opportunity for early detection and planning of definitive intervention. This may allow for more liver parenchymal-sparing techniques in the form of metastectomies and also improve the success of redo-hepatectomies[42]. The earlier the diagnosis is made, the more likely it is going to be resectable[43].

The lungs are the second most common location for metastatic spread of colorectal cancer[44]. It occurs in 5%-15% of patients and not all of these have concurrent liver metastases[45]. Only 4.1% of patients with synchronous pulmonary metastases are resectable, whereas 14.8% of patients with metachronous pulmonary metastases are resectable[46] with a 36%-40% 5-year survival rate in resected patients[47]. In our study, 14 (5.2%) patients developed lung metastases in the follow-up period with a median survival period of 2.17 years. Nodal status and Dukes stage were predictive of early lung recurrence on univariate analysis but not multivariate analysis. This result should be treated with caution due to the limited number of patients who developed lung metastases. This is in contrast to a similar study by Kim *et al*[48] that reported a 3 year overall survival rate of 54.6%, although their study included 105 patients. Our study only contained 14 patients who developed lung metastases and it is possible that increased survival may have occurred with more patients in this category.

Negative predictive features when considering a patient for pulmonary metastatectomy follow a similar pattern to metastasis elsewhere and include an unresectable primary tumour, extra-pulmonary metastases, resection margins (R1/2) and mediastinal lymph node disease[49]. Blackmon *et al*[50] concluded that more than three lung metastases present at the first metastatectomy and a preoperative disease free survival of less than three years predicts recurrence. This suggests that early pickup of metastases means more patients can be considered for a successful resection. However, due to a small number of patients with lung disease in our cohort, no meaningful analyses of multiple clinical and pathological factors could be carried out.

The value of early detection of metastatic disease in offering an absolute reduction in mortality is clear[15]. Predictive tools can aid the clinician in identifying patients at higher risk of early metachronous metastasis as not all patients will benefit from aggressive surveillance. Our results suggest that there may be a subgroup of patients who would benefit from more intensive follow-up. As most tumours recur within the first three years after resection[34,51], it is imperative that the focus on follow-up occurs during this time frame. However, there is a lack of specific guidance for patients who may be at increased risk.

Current surveillance protocols consist of a combination of CEA testing, CT scans, and colonoscopy. Emergency surgery and the presence of specific histological features can inform the selection of patients

at high-risk of early recurrence and should be factored into surveillance strategies. There is a substantial range in intensity of follow-up with the United Kingdom having noticeably less intensive follow-up in comparison to American collaborators[4,21,22]. A recent United Kingdom study of 1202 participants who had undergone curative surgery for primary colorectal cancer found no survival benefit from combined intensive monitoring groups compared to follow-up only if symptoms recurred[52]. This study was a randomised trial and so highlights the need to target the patients that would benefit from an intensive follow-up regimen. This has benefits for all stakeholders; earlier detection of recurrence in high-risk patients and a reduction of unnecessary investigations in the low-risk group. Exciting developments in the availability of other prognostic markers in the future may enhance the efficacy of a risk-adapted follow-up strategy[53]. The overexpression of vascular endothelial growth factor[54] and interleukin-8[55] in colorectal carcinoma cells are two such examples.

Our study is limited, foremost by the retrospective approach of data gathering using the hospital coding process. The weaknesses of such a design are well known[56]. Secondly, relevant data on neoadjuvant and adjuvant therapies and cause of death was not available to afford reliable assessment of correlation. More patients in the emergency resection group received postoperative adjuvant therapies which may have been the result of a higher tumour stage (T4), early recurrence disease or palliative treatment. Indication for post-operative therapies and cause of death outcomes were not collected and survival was calculated on basis of all-cause mortality. The majority of recurrent disease occurred in the liver, leading to more reliable statistical conclusions here. However, this was limited in number for both lung and local recurrent disease reflecting differences in association of histological and clinical predictive features. Furthermore, low event rates in neo-adjuvant therapies and positive resection margins, missing data on total number of lymph nodes harvested, limited reliable evaluation of these factors in overall outcomes.

Although emergency presentation in the form of obstruction continues to represent a significant proportion of patients with initial diagnosis, self-expanding colonic metal stents are likely to play an increasingly important role in improving immediate postoperative outcomes, stoma rates and long-term outcomes without adverse oncological outcomes[28]. Proven survival benefit from primary and redo liver resections in isolated disease and emerging technologies for palliative control of local and distant metastatic disease mean that predictive clinicopathological markers could be used in a more intensive, targeted surveillance strategy to identify more patients with early recurrence. Emergency resection, tumour stage, lymphovascular invasion and lymph node ratio > 0.015 represent a high-risk of recurrent disease and can inform surveillance strategies to enable early interventions.

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer is the fourth most common cancer in the United Kingdom with over 40000 cases diagnosed each year. Despite the widespread use of screening programs, a large number of cases are diagnosed in the acute or urgent setting with adverse post-operative mortality, disease-free and overall long-term survival.

Research motivation

A large proportion of patients with colorectal cancer are diagnosed in the acute setting with an emergency presentation (26%) or following an urgent 2-wk referral (27%). Compared to elective resection, emergency surgery is associated with adverse postoperative outcomes (post-operative mortality 4.6% *vs* 16%), disease-free and overall long-term survival. The basis for this is multifactorial and may include altered physiology, immune-suppression, adverse tumour biology, advanced disease, peri-operative complications and lower progression to adjuvant therapies.

Research objectives

Adverse predictive factors for survival in colorectal cancer include emergency presentation with obstruction or perforation and histo-pathological features such as T4 disease, advanced nodal disease. Lymph node ratio offers to a new representation of nodal disease. Although it is affected directly but the lymph node yield, its utility as a predictive tool for recurrent disease has not been evaluated. We aimed to identify clinical and histological predictive factors for early recurrence disease and pattern to inform surveillance strategies and aid in early detection.

Research methods

Following institutional approval, a retrospective study of clinical and histo-pathological parameters was carried out to study patterns of recurrence and survival in consecutive patients undergoing elective and emergency resection for colorectal cancer over an eight-year study period.

Research results

Outcomes were evaluated in 266 consecutive patients following curative surgery with a median follow-up of 7.9 years. The proportion of patients undergoing emergency resection was 13.2%. Recurrent disease was detected in 67 patients (25.2%) during follow-up with the majority identified early within 3 years (82.1%). Liver was the predominant site of metastatic disease (73.1%). Emergency resection (OR = 3.60, $P = 0.001$), T4 stage (OR = 4.33, $P < 0.001$) and lymphovascular invasion (LVI) (OR = 2.37, $P = 0.032$) were associated with higher risk of recurrent disease. Emergency resection, T4 disease and a high lymph node ratio (LNR) were strong independent predictors of adverse long-term survival.

Research conclusions

Our study reaffirms the independent predictive potential of histological and clinical features for recurrent disease in patients undergoing emergency resection for colorectal cancer. Furthermore, it introduces the independent utility of lymph node ratio (LNR) alongside T stage and lympho-vascular invasion in identifying patients with high risk of recurrent disease.

Research perspectives

Modified surveillance strategies should be evaluated in presence of adverse clinical and histological factors to improve early detection of recurrent disease in high-risk patients to offset adverse disease-free and overall long-term survival.

REFERENCES

1 **Cancer Research UK.** Bowel cancer incidence statistics. [accessed 2014 Jun]. Available from: URL: http://www.cancerresearchuk.

org/cancer-info/cancerstats/types/bowel/incidence/
2 **Tan EK**, Ooi LL. Colorectal cancer liver metastases - understanding the differences in the management of synchronous and metachronous disease. *Ann Acad Med Singapore* 2010; **39**: 719-715 [PMID: 20957308]
3 **Garden OJ**, Rees M, Poston GJ, Mirza D, Saunders M, Ledermann J, Primrose JN, Parks RW. Guidelines for resection of colorectal cancer liver metastases. *Gut* 2006; **55** Suppl 3: iii1-iii8 [PMID: 16835351 DOI: 10.1136/gut.2006.098053]
4 **Meyerhardt JA**, Mangu PB, Flynn PJ, Korde L, Loprinzi CL, Minsky BD, Petrelli NJ, Ryan K, Schrag DH, Wong SL, Benson AB 3rd; American Society of Clinical Oncology. Follow-up care, surveillance protocol, and secondary prevention measures for survivors of colorectal cancer: American Society of Clinical Oncology clinical practice guideline endorsement. *J Clin Oncol* 2013; **31**: 4465-4470 [PMID: 24220554 DOI: 10.1200/JCO.2013.50.7442]
5 **Scheele J**, Stangl R, Altendorf-Hofmann A. Hepatic metastases from colorectal carcinoma: impact of surgical resection on the natural history. *Br J Surg* 1990; **77**: 1241-1246 [PMID: 2253003 DOI: 10.1002/bjs.1800771115]
6 **Nozawa H**, Sunami E, Nakajima J, Nagawa H, Kitayama J. Synchronous and metachronous lung metastases in patients with colorectal cancer: A 20-year monocentric experience. *Exp Ther Med* 2012; **3**: 449-456 [PMID: 22969910 DOI: 10.3892/etm.2011.443]
7 **Landmann RG**, Weiser MR. Surgical management of locally advanced and locally recurrent colon cancer. *Clin Colon Rectal Surg* 2005; **18**: 182-189 [PMID: 20011301 DOI: 10.1055/s-2005-916279]
8 **Read TE**, Mutch MG, Chang BW, McNevin MS, Fleshman JW, Birnbaum EH, Fry RD, Caushaj PF, Kodner IJ. Locoregional recurrence and survival after curative resection of adenocarcinoma of the colon. *J Am Coll Surg* 2002; **195**: 33-40 [PMID: 12113543 DOI: 10.1016/S1072-7515(02)01224-3]
9 **Pastorino U**, Buyse M, Friedel G, Ginsberg RJ, Girard P, Goldstraw P, Johnston M, McCormack P, Pass H, Putnam JB Jr; International Registry of Lung Metastases. Long-term results of lung metastasectomy: prognostic analyses based on 5206 cases. *J Thorac Cardiovasc Surg* 1997; **113**: 37-49 [PMID: 9011700 DOI: 10.1016/S0022-5223(97)70397-0]
10 **Nordlinger B**, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, Bechstein WO, Primrose JN, Walpole ET, Finch-Jones M, Jaeck D, Mirza D, Parks RW, Collette L, Praet M, Bethe U, Van Cutsem E, Scheithauer W, Gruenberger T; EORTC Gastro-Intestinal Tract Cancer Group; Cancer Research UK; Arbeitsgruppe Lebermetastasen und-tumoren in der Chirurgischen Arbeitsgemeinschaft Onkologie (ALM-CAO); Australasian Gastro-Intestinal Trials Group (AGITG); Fédération Francophone de Cancérologie Digestive (FFCD). Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. *Lancet* 2008; **371**: 1007-1016 [PMID: 18358928 DOI: 10.1016/S0140-6736(08)60455-9]
11 **Bruinvels DJ**, Stiggelbout AM, Kievit J, van Houwelingen HC, Habbema JD, van de Velde CJ. Follow-up of patients with colorectal cancer. A meta-analysis. *Ann Surg* 1994; **219**: 174-182 [PMID: 8129488 DOI: 10.1097/00000658-199402000-00009]
12 **House MG**, Ito H, Gönen M, Fong Y, Allen PJ, DeMatteo RP, Brennan MF, Blumgart LH, Jarnagin WR, D'Angelica MI. Survival after hepatic resection for metastatic colorectal cancer: trends in outcomes for 1,600 patients during two decades at a single institution. *J Am Coll Surg* 2010; **210**: 744-752, 752-755 [PMID: 20421043 DOI: 10.1016/j.jamcollsurg.2009.12.040]
13 **Jeffery M**, Hickey BE, Hider PN. Follow-up strategies for patients treated for non-metastatic colorectal cancer. *Cochrane Database Syst Rev* 2007; CD002200 [PMID: 17253476 DOI: 10.1002/14651858.CD002200.pub2]
14 **Figueredo A**, Rumble RB, Maroun J, Earle CC, Cummings B, McLeod R, Zuraw L, Zwaal C; Gastrointestinal Cancer Disease Site Group of Cancer Care Ontario's Program in Evidence-based Care. Follow-up of patients with curatively resected colorectal cancer: a practice guideline. *BMC Cancer* 2003; **3**: 26 [PMID: 14529575 DOI: 10.1186/1471-2407-3-26]
15 **Renehan AG**, Egger M, Saunders MP, O'Dwyer ST. Impact on survival of intensive follow up after curative resection for colorectal cancer: systematic review and meta-analysis of randomised trials. *BMJ* 2002; **324**: 813 [PMID: 11934773 DOI: 10.1136/bmj.324.7341.813]
16 **Renehan AG**, O'Dwyer ST, Whynes DK. Cost effectiveness analysis of intensive versus conventional follow up after curative resection for colorectal cancer. *BMJ* 2004; **328**: 81 [PMID: 14715603 DOI: 10.1136/bmj.328.7431.81]
17 **National Cancer Intelligence Network data briefing.** Routes to diagnosis. NCIN, November 2010. [accessed 2015 Mar]. Available from: URL: http://www.ncin.org.uk/databriefings
18 **Wong SK**, Jalaludin BB, Morgan MJ, Berthelsen AS, Morgan A, Gatenby AH, Fulham SB. Tumor pathology and long-term survival in emergency colorectal cancer. *Dis Colon Rectum* 2008; **51**: 223-230 [PMID: 18097722 DOI: 10.1007/s10350-007-9094-2]
19 **Morris EJ**, Taylor EF, Thomas JD, Quirke P, Finan PJ, Coleman MP, Rachet B, Forman D. Thirty-day postoperative mortality after colorectal cancer surgery in England. *Gut* 2011; **60**: 806-813 [PMID: 21486939 DOI: 10.1136/gut.2010.232181]
20 **Hogan J**, Samaha G, Burke J, Chang KH, Condon E, Waldron D, Coffey JC. Emergency presenting colon cancer is an independent predictor of adverse disease-free survival. *Int Surg* 2015; **100**: 77-86 [PMID: 25594643 DOI: 10.9738/INTSURG-D-13-00281.1]
21 **Vallejo R**, Hord ED, Barna SA, Santiago-Palma J, Ahmed S. Perioperative immunosuppression in cancer patients. *J Environ Pathol Toxicol Oncol* 2003; **22**: 139-146 [PMID: 14533877 DOI: 10.1615/JEnvPathToxOncol.v22.i2.70]
22 **Ghazi S**, Berg E, Lindblom A, Lindforss U; Low-Risk Colorectal Cancer Study Group. Clinicopathological analysis of colorectal cancer: a comparison between emergency and elective surgical cases. *World J Surg Oncol* 2013; **11**: 133 [PMID: 23758762 DOI: 10.1186/1477-7819-11-133]
23 **Baca B**, Beart RW Jr, Etzioni DA. Surveillance after colorectal cancer resection: a systematic review. *Dis Colon Rectum* 2011; **54**: 1036-1048 [PMID: 21730795 DOI: 10.1007/DCR.0b013e31820db364]
24 **The Association of Coloproctologists of Great Britain and Ireland**. Guidelines for the Management of Colorectal Cancer. Third edition, 2007.
25 **Benson AB**, Arnoletti P, Bekaii-Saab T, Chan E, Chen YJ, Choti MA, Cooper HS, Dilawari RA, Engstrom PF, Enzinger PC, Fleshman JW, Fuchs CS, Grem JL, Knol JA, Leong LA, Lin E, May KS, Mulcahy MF, Murphy K, Rohren E, Ryan DP, Saltz L, Sharma S, Shibata D, Skibber JM, Small W, Sofocleous CT, Venook AP, Willett C. Clinical practice and guidelines in oncology (National Comprehensive Cancer Network Guidelines) Colon Cancer NCCN Version 3, 2014
26 **Scott N,** Hill J, Smith J, Kelly S, Fearnhead N. National Bowel Cancer Audit Annual Report. Association of Coloproctologist of Great Britain and Ireland. 2013
27 **Tan CJ**, Dasari BV, Gardiner K. Systematic review and meta-analysis of randomized clinical trials of self-expanding metallic stents as a bridge to surgery versus emergency surgery for malignant left-sided large bowel obstruction. *Br J Surg* 2012; **99**: 469-476 [PMID: 22261931 DOI: 10.1002/bjs.8689]
28 **Hill J,** Kay C, Morton D, Magill L, Handley K, Gray R. CREST Trial Collaborative Group CREST: Randomised phase III study of stenting as a bridge to surgery in obstructing colorectal cancer-Results of the UK ColoRectal Endoscopic Stenting Trial (CREST). *J Clin Oncol* 2016; **34** (suppl): Abstr 3507
29 **Liang JL**, Wan DS, Pan ZZ, Zhou ZW, Chen G, Li LR, Lu ZH, Wu XJ. [Multivariate regression analysis of recurrence following curative surgery for colorectal cancer]. *Ai Zheng* 2004; **23**: 564-567 [PMID: 15142455]
30 **Keum MA**, Lim SB, Kim SA, Yoon YS, Kim CW, Yu CS, Kim JC. Clinicopathologic factors affecting recurrence after curative surgery

for stage I colorectal cancer. *J Korean Soc Coloproctol* 2012; **28**: 49-55 [PMID: 22413082 DOI: 10.3393/jksc.2012.28.1.49]
31 **Hellinger MD**, Santiago CA. Reoperation for recurrent colorectal cancer. *Clin Colon Rectal Surg* 2006; **19**: 228-236 [PMID: 20011326 DOI: 10.1055/s-2006-956445]
32 **Huh JW**, Kim CH, Lim SW, Kim HR, Kim YJ. Early recurrence in patients undergoing curative surgery for colorectal cancer: is it a predictor for poor overall survival? *Int J Colorectal Dis* 2013; **28**: 1143-1149 [PMID: 23503665 DOI: 10.1007/s00384-013-1675-z]
33 **Lim SB**, Yu CS, Jang SJ, Kim TW, Kim JH, Kim JC. Prognostic significance of lymphovascular invasion in sporadic colorectal cancer. *Dis Colon Rectum* 2010; **53**: 377-384 [PMID: 20305435 DOI: 10.1007/DCR.0b013e3181cf8ae5]
34 **Sargent DJ**, Wieand HS, Haller DG, Gray R, Benedetti JK, Buyse M, Labianca R, Seitz JF, O'Callaghan CJ, Francini G, Grothey A, O'Connell M, Catalano PJ, Blanke CD, Kerr D, Green E, Wolmark N, Andre T, Goldberg RM, De Gramont A. Disease-free survival versus overall survival as a primary end point for adjuvant colon cancer studies: individual patient data from 20,898 patients on 18 randomized trials. *J Clin Oncol* 2005; **23**: 8664-8670 [PMID: 16260700 DOI: 10.1200/JCO.2005.01.6071]
35 **Bar-Gil Shitrit A**, Shitrit D, Bakal I, Braverman D, Kramer MR. Endobronchial metastases from colon cancer without liver metastases: report of eight cases. *Dis Colon Rectum* 2007; **50**: 1087-1089 [PMID: 17347902 DOI: 10.1007/s10350-006-0880-z]
36 **Pietra N**, Sarli L, Costi R, Ouchemi C, Grattarola M, Peracchia A. Role of follow-up in management of local recurrences of colorectal cancer: a prospective, randomized study. *Dis Colon Rectum* 1998; **41**: 1127-1133 [PMID: 9749496 DOI: 10.1007/BF02239434]
37 **Morris EJ**, Forman D, Thomas JD, Quirke P, Taylor EF, Fairley L, Cottier B, Poston G. Surgical management and outcomes of colorectal cancer liver metastases. *Br J Surg* 2010; **97**: 1110-1118 [PMID: 20632280 DOI: 10.1002/bjs.7032]
38 **de Jong MC**, Pulitano C, Ribero D, Strub J, Mentha G, Schulick RD, Choti MA, Aldrighetti L, Capussotti L, Pawlik TM. Rates and patterns of recurrence following curative intent surgery for colorectal liver metastasis: an international multi-institutional analysis of 1669 patients. *Ann Surg* 2009; **250**: 440-448 [PMID: 19730175 DOI: 10.1097/SLA.0b013e3181b4539b]
39 **Fernandez FG**, Drebin JA, Linehan DC, Dehdashti F, Siegel BA, Strasberg SM. Five-year survival after resection of hepatic metastases from colorectal cancer in patients screened by positron emission tomography with F-18 fluorodeoxyglucose (FDG-PET). *Ann Surg* 2004; **240**: 438-447; discussion 447-450 [PMID: 15319715 DOI: 10.1097/01.sla.0000138076.72547.b1]
40 **Jamison RL**, Donohue JH, Nagorney DM, Rosen CB, Harmsen WS, Ilstrup DM. Hepatic resection for metastatic colorectal cancer results in cure for some patients. *Arch Surg* 1997; **132**: 505-510; discussion 511 [PMID: 9161393 DOI: 10.1001/archsurg.1997.01430290051008]
41 **Malik HZ**, Hamady ZZ, Adair R, Finch R, Al-Mukhtar A, Toogood GJ, Prasad KR, Lodge JP. Prognostic influence of multiple hepatic metastases from colorectal cancer. *Eur J Surg Oncol* 2007; **33**: 468-473 [PMID: 17097260 DOI: 10.1016/j.ejso.2006.09.030]
42 **Allen PJ**, Jarnagin WR. Current status of hepatic resection. *Adv Surg* 2003; **37**: 29-49 [PMID: 12953626]
43 **Adam R**, Lucidi V, Bismuth H. Hepatic colorectal metastases: methods of improving resectability. *Surg Clin North Am* 2004; **84**: 659-671 [PMID: 15062667 DOI: 10.1016/j.suc.2003.12.005]
44 **McCormack PM**, Attiyeh FF. Resected pulmonary metastases from colorectal cancer. *Dis Colon Rectum* 1979; **22**: 553-556 [PMID: 527448 DOI: 10.1007/BF02587003]
45 **Tan KK**, Lopes Gde L Jr, Sim R. How uncommon are isolated lung metastases in colorectal cancer? A review from database of 754 patients over 4 years. *J Gastrointest Surg* 2009; **13**: 642-648 [PMID: 19082673 DOI: 10.1007/s11605-008-0757-7]
46 **Mitry E**, Guiu B, Cosconea S, Jooste V, Faivre J, Bouvier AM. Epidemiology, management and prognosis of colorectal cancer with lung metastases: a 30-year population-based study. *Gut* 2010; **59**: 1383-1388 [PMID: 20732912 DOI: 10.1136/gut.2010.211557]
47 **Fiorentino F**, Hunt I, Teoh K, Treasure T, Utley M. Pulmonary metastasectomy in colorectal cancer: a systematic review and quantitative synthesis. *J R Soc Med* 2010; **103**: 60-66 [PMID: 20118336 DOI: 10.1258/jrsm.2009.090299]
48 **Kim CH**, Huh JW, Kim HJ, Lim SW, Song SY, Kim HR, Na KJ, Kim YJ. Factors influencing oncological outcomes in patients who develop pulmonary metastases after curative resection of colorectal cancer. *Dis Colon Rectum* 2012; **55**: 459-464 [PMID: 22426271 DOI: 10.1097/DCR.0b013e318246b08d]
49 **Treasure T**, Milošević M, Fiorentino F, Macbeth F. Pulmonary metastasectomy: what is the practice and where is the evidence for effectiveness? *Thorax* 2014; **69**: 946-949 [PMID: 24415715 DOI: 10.1136/thoraxjnl-2013-204528]
50 **Blackmon SH**, Stephens EH, Correa AM, Hofstetter W, Kim MP, Mehran RJ, Rice DC, Roth JA, Swisher SG, Walsh GL, Vaporciyan AA. Predictors of recurrent pulmonary metastases and survival after pulmonary metastasectomy for colorectal cancer. *Ann Thorac Surg* 2012; **94**: 1802-1809 [PMID: 23063195 DOI: 10.1016/j.athoracsur.2012.07.014]
51 **Berman JM**, Cheung RJ, Weinberg DS. Surveillance after colorectal cancer resection. *Lancet* 2000; **355**: 395-399 [PMID: 10665570 DOI: 10.1016/S0140-6736(99)06552-6]
52 **Primrose JN**, Perera R, Gray A, Rose P, Fuller A, Corkhill A, George S, Mant D; FACS Trial Investigators. Effect of 3 to 5 years of scheduled CEA and CT follow-up to detect recurrence of colorectal cancer: the FACS randomized clinical trial. *JAMA* 2014; **311**: 263-270 [PMID: 24430319 DOI: 10.1001/jama.2013.285718]
53 **Rose J**, Augestad KM, Cooper GS. Colorectal cancer surveillance: what's new and what's next. *World J Gastroenterol* 2014; **20**: 1887-1897 [PMID: 24587668 DOI: 10.3748/wjg.v20.i8.1887]
54 **Toiyama Y**, Inoue Y, Saigusa S, Okugawa Y, Yokoe T, Tanaka K, Miki C, Kusunoki M. Gene expression profiles of epidermal growth factor receptor, vascular endothelial growth factor and hypoxia-inducible factor-1 with special reference to local responsiveness to neoadjuvant chemoradiotherapy and disease recurrence after rectal cancer surgery. *Clin Oncol* (R Coll Radiol) 2010; **22**: 272-280 [PMID: 20117921 DOI: 10.1016/j.clon.2010.01.001]
55 **Lurje G**, Zhang W, Schultheis AM, Yang D, Groshen S, Hendifar AE, Husain H, Gordon MA, Nagashima F, Chang HM, Lenz HJ. Polymorphisms in VEGF and IL-8 predict tumor recurrence in stage III colon cancer. *Ann Oncol* 2008; **19**: 1734-1741 [PMID: 18550579 DOI: 10.1093/annonc/mdn368]
56 **Audit commission.** PbR Data assurance Framework 2007/2008. Audit Commission London

P- Reviewer: Mayol J, Nishida T, Zhu X **S- Editor**: Kong JX
L- Editor: A **E- Editor**: Li D

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: http://www.f6publishing.com

World J Gastrointest Pathophysiol 2018 February 15; 9(1): 18-27

DOI: 10.4291/wjgp.v9.i1.18

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Observational Study

Abundance of *Enterobacteriaceae* in the colon mucosa in diverticular disease

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Author contributions: Roth B, Erlanson-Albertsson C, Molin G, Toth E, and Ohlsson B designed the study; Toth E was responsible for the endoscopic examinations and biopsy sampling; Linninge C and Roth B performed the microbial examinations; Ohlsson B wrote the initial draft of the manuscript; all authors contributed to the intellectual criticism of the manuscript and accepted the final version.

Supported by Development Foundation of Region Skåne (BO), No. F2014/354.

Institutional review board statement: The study was reviewed and approved by the Ethics Review Board of Lund University (2013/903).

Informed consent statement: The subjects provided written, informed consent prior to study enrollment.

Conflict-of-interest statement: The authors have no conflicts of interests to declare.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at bodil.ohlsson@med.lu.se. Consent from participants was not obtained for data sharing, but the presented data are anonymized and risk of identification is low.

Manuscript source: Unsolicited manuscript

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Received: August 30, 2017
Peer-review started: September 5, 2017
First decision: October 12, 2017
Revised: November 10, 2017
Accepted: December 6, 2017
Article in press: December 6, 2017
Published online: February 15, 2018

Abstract

AIM

To compare gut bacterial diversity and amount of *Enterobacteriaceae* in colonic mucosa between patients with and without diverticular disease (DD).

METHODS

Patients in a stable clinical condition with planned elective colonoscopy were included. Blood samples and colon mucosa biopsies were collected at the

colonoscopy. Study questionnaires including questions about gastrointestinal symptoms were completed by the patients and physicians. DNA from mucosa samples was isolated and the amount of *Enterobacteriaceae* was estimated using PCR assay. Terminal restriction fragment length polymorphism was applied to assess microbial diversity. Diversity was estimated by calculations of richness (number of terminal restriction fragments) and Shannon-Wiener and Simpson's indices.

RESULTS

A total of 51 patients were included, 16 patients with DD [68 (62-76) years] and 35 controls [62 (40-74) years] without any diverticula. Patients with DD had significantly higher levels of *Enterobacteriaceae* than those without DD (P = 0.043), and there was an inverse relationship between the amount of *Enterobacteriaceae* and the Simpson's index (rs = -0.361, P = 0.033) and the Shannon-Wiener index (rs = -0.299, P = 0.081). The Simpson's index (P = 0.383), Shannon-Wiener index (P = 0.401) or number of restrictions fragments (P = 0.776) did not differ between DD and controls. The majority of patients experienced gastrointestinal symptoms, and 22 patients (43.1%) fulfilled the criteria for irritable bowel syndrome, with no difference between the groups (P = 0.212). Demography, socioeconomic status, lifestyle habits, inflammatory biomarkers, or symptoms were not related to the amount of *Enterobacteriaceae* or bacterial diversity.

CONCLUSION

Patients with DD had higher amount of *Enterobacteriaceae* in the colon mucosa compared to patients without diverticula.

Key words: Bacterial diversity; Diverticular disease; *Enterobacteriaceae*; Gut microbiota; Irritable bowel syndrome

Core tip: Colon mucosa biopsies were collected from consecutive patients (n = 51) at the time of elective colonoscopy. Patients were grouped into patients with diverticular disease (DD) (n = 16) and controls without any diverticula (n = 35). The amount of *Enterobacteriaceae* and bacterial diversity were analyzed. Patients with DD had significantly higher levels of *Enterobacteriaceae* than controls (P = 0.043). Bacterial diversity did not differ between groups. All but 8 patients exhibited some kind of gastrointestinal symptoms, and 22 patients (43.1%) fulfilled the criteria for irritable bowel syndrome, without difference between groups (P = 0.212). Demography, socioeconomic status, lifestyle habits, inflammatory parameters, or gastrointestinal symptoms did not affect the gut microbiota examined.

Linninge C, Roth B, Erlanson-Albertsson C, Molin G, Toth E, Ohlsson B. Abundance of *Enterobacteriaceae* in the colon mucosa in diverticular disease. *World J Gastrointest Pathophysiol* 2018; 9(1): 18-27 Available from: URL: http://www.wjgnet.com/2150-5330/full/v9/i1/18.htm DOI: http://dx.doi.org/10.4291/wjgp.v9.i1.18

INTRODUCTION

Diverticular disease (DD) is a common gastrointestinal disease of unknown etiology. The symptoms of DD are similar with symptoms of irritable bowel syndrome (IBS)[1], *e.g*., abdominal pain, bloating and altered bowel habits, and are present in 10%-25% of subjects[2]. About 1.5%-4% of patients with DD develop diverticulitis at some time during their lives[3,4]. An acute attack of diverticulitis may lead to chronic symptoms called post-diverticulitis IBS, in analogy with post-infectious IBS observed after an acute attack of gastroenteritis[5,6]. The hypothesis behind IBS development is that low-grade inflammation and/or altered intestinal gut microbiota in DD may contribute to visceral hypersensitivity and dysmotility with ensuing symptoms[7,8].

The gut microbiota is discussed as important for the etiology and pathophysiology in a wide range of diseases. Bacterial diversity is higher in lean compared to obese individuals, and in healthy states compared to unhealthy states, and some bacterial groups, *e.g*., *Enterobacteriaceae,* are associated with over-weight and inflammation[9-11]. The family *Enterobacteriaceae* is commonly found in the gut ecosystem, where *Escherichia coli* is the most abundant species of the family[9]. Low bacterial diversity and increased levels of *Enterobacteriaceae/Escherichia coli* have been linked to inflammatory bowel disease (IBD) in humans[12-14]. The findings of abundance of *Enterobacteriaceae/Escherichia coli* in experimental animal models of intestinal inflammation[15], and the ability of these bacteria to induce colitis[16], have strengthened the hypothesis that these bacteria are of importance in the etiology of IBD.

Only a few studies have been performed regarding microbial composition in DD. Recently, lower amounts of *Enterobacteriaceae* were found in the colon mucosa of DD patients compared with healthy controls[17], whereas higher amounts of *Akkermansia* and no difference in the *Escherichia coli* subgroup were found in feces in another DD cohort[18].

The primary aim of the present study was to compare the level of the large Gram-negative bacterial family *Enterobacteriaceae* and gut bacterial diversity in colon mucosa between consecutive patients diagnosed with DD and patients with normal endoscopic findings. Secondary aims were to evaluate the influence of demography, socioeconomic status, lifestyle habits, inflammatory parameters and gastrointestinal symptoms on the gut microbiota.

MATERIALS AND METHODS

Study population and study design

All consecutive patients referred to elective colonoscopy at the Department of Endoscopy, Skåne University Hospital, Malmö, were invited to participate in the study. All patients were in a stabile clinical condition, and no one suffered from any acute inflammation, such as diverticulitis. The only exclusion criteria were age of ⩽ 18 years and inability to understand the Swedish language. The patients were informed in oral and written communications at the arrival to the Department the day of examination. If they agreed to participate, they had to complete a study questionnaire about demography, socioeconomic status, lifestyle habits, family history and medical history, the Visual Analog Scale for Irritable Bowel Syndrome (VAS-IBS), and a nutrition questionnaire to analyze dietary habits. The colonoscopy was performed according to clinical routines. Four different mucosa biopsies were obtained from the mid part of the colon descendens. Samples were stored at -80 °C until the gut microbiota was analyzed by quantitative polymerase chain reaction (qPCR) and terminal restriction fragment length polymorphism (T-RFLP). Blood samples were collected according to clinical routines and analyzed at the Department of Clinical Chemistry. A study protocol was completed by the physician about clinical findings and histopathological diagnoses. The patients were divided into two groups depending on the colonoscopy finding: patients with DD, and patients without any diverticula who served as controls.

Tissue sampling

The patients were examined by colonoscopy according to clinical routines after prior laxation with Laxabon® (potassium chloride and macrogol; BioPhausia, Stockholm, Sweden). At the end of the colonoscopy, when the clinical examination was completed, four different mucosa biopsies were obtained from intact, inter-diverticular mucosa in the mid part of the colon descendens. This location was chosen since the left colon is the region most often affected by diverticula and is more accessible than the right colon. The biopsies were immediately frozen in liquid nitrogen and kept frozen at -80 °C until analysis. Histopathological examination was performed on separate mucosa samples when IBD had to be excluded or verified.

Questionnaires

Study questionnaire: The questionnaire included questions on age, body mass index (BMI), family history, lifestyle habits, educational achievement, occupation, civil status, circumstances concerning delivery and breast-feeding, place of birth and moving patterns, and medical history. The patients had to answer whether they had been diagnosed with celiac disease, IBD, lactose intolerance, reflux or ulcer. They were asked whether they experienced gastrointestinal symptoms which fulfilled the Rome IV criteria of functional dyspepsia or IBS[19,20]. This questionnaire was in structure and design similar to questionnaires used by other large current population-based and on-going screening projects in Sweden (*i.e.*, LifeGene, EpiHealth, BIG-3, SCAPIS).

VAS-IBS: The VAS-IBS was used to investigate gastrointestinal complaints in the study groups. VAS-IBS is a validated, self-rating questionnaire for estimation of the most common gastrointestinal complaints experienced during the last 2 wk[21]. This questionnaire has also been validated for estimation of symptoms over time[22]. The five items measured in the VAS-IBS address the symptoms of abdominal pain, diarrhea, constipation, bloating and flatulence, and nausea and vomiting. These items were measured on a scale from 0-100, where 0 represented severe problems and 100 represented a complete lack of problems. Whether the patient suffered from symptoms or not, was defined as a score above the median values in healthy subjects[22].

Food questionnaire: The questionnaire included questions about dietary intake each meal in the form of red meat, fish or vegetables, making it possible to estimate dietary patterns. The number of days per week for intake of juice, coffee/tea, milk, sour milk, muesli, berries and fruit, marmalade, bread, cheese, ham and egg at breakfast, or snack were filled in. The participants were asked whether the lunch and dinner were homemade, or whether the participant had a lunch or dinner at a restaurant or a frozen precooked meal.

Microbial analyses

DNA extraction: Three of the four mucosa samples, mean weight 15 ± 0.6 mg, were used for DNA extraction. DNA was isolated and purified by EZ1 Advanced XL (EZ1 DNA Tissue Kit and Bacteria Card; Qiagen, Hilden, Germany)[10,23].

qPCR: The amount of *Enterobacteriaceae* was estimated using a qPCR assay according to Karlsson *et al*[10]. Primers used for the qPCR assay have been used and published previously[24,25]. The detection limit was 10^2 genes/reaction. For standard curves, 10-fold dilution series of the target DNA were made in EB buffer (Qiagen). Number of bacteria was expressed as $\log_{10}$ 16S rRNA genes/g feces.

Microbial diversity: T-RFLP was applied to assess the microbial diversity, as previously described[26]. Thresholds for internal standard and terminal restriction fragments (T-RFs) were set to 5 and 15 fluorescence units, respectively.

Calculations: Microbial diversity was estimated by calculation of richness (number of T-RFs) and Shannon-

Wiener and Simpson's diversity indices as described by Karlsson *et al*[10], with the exception that T-RFs within 40-580 base pairs were included in the T-RFLP profile analysis and calculation. The diversity indices take into account both richness and evenness when considering the relative abundance of bacterial groups. Both indices are commonly used to assess microbial diversity[27]. Samples below the limit of detection (in qPCR) were replaced by the limit of detection for statistical analysis.

Patient categorization

Depending on presence or absence of diverticula, the included patients were divided into patients with DD or control patients. The control patients either exhibited normal macroscopic endoscopic and microscopic histopathological findings or presence of benign polyps. The group categorization was performed independent of gastrointestinal symptoms. All patients with IBD or malignancy were excluded from the study. The diagnosis of IBD was set when the patients fulfilled the criteria for Crohn's disease, ulcerative colitis or microscopic colitis, *i.e.,* clinical and endoscopic findings in addition to inflammation at the histopathological examination, in accordance to the diagnoses criteria[28].

Statistical analysis

The statistical calculations were performed using the SPSS software, version 24.0 (Armonk, NY, United States). Non-parametric tests were used because of the low number of participants in each group and the skewed distribution of the values of VAS-IBS. Comparisons of continuous variables between groups were performed by either Mann-Whitney *U* test or Kruskal-Wallis test. Fisher's exact test was used for dichotomous variables, and Spearman's correlation test was used for correlations between parameters. Values are presented in median and interquartile ranges (IQRs) or number and percentage. $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

In total, 77 patients were invited to participate in the study. Nineteen patients denied to participate and 58 patients were included. Six patients were later excluded since they fulfilled the criteria for IBD, and one because of colon malignancy. Finally, 51 patients were included in the present study, 16 with DD and 35 controls without organic changes visible at the colonoscopy or at the histopathological examination ($n = 12$), except non-malignant polyps ($n = 23$). The reasons for referral to colonoscopy were presence of gastrointestinal symptoms which rendered a colonoscopy to exclude IBD, malignancy or DD ($n = 17$), follow-up after previous resection of polyps ($n = 17$), rectal bleeding ($n = 11$), screening for cancer due to heredity ($n = 4$), or perforation to the urinary tract ($n = 2$). Only one subject in the DD group had a history of verified acute diverticulitis.

There was an equal sex distribution in the groups. Subjects without DD were slightly older than controls [68 (62-76) years *vs* 62 (40-74) years, $P = 0.072$], which may explain that more DD patients than controls had completed primary school as the highest education level. Age differences may also explain the lower degree of physical activity in the DD group. A few patients in both groups had been treated with antibiotics during the last 6 mo (Table 1). The moving patterns did not differ between groups. Sporadic cases of heart and lung diseases were found in both groups (data not shown).

Gastrointestinal symptoms

Altogether, 22 patients (43.1%) fulfilled the Rome IV criteria for IBS. The prevalence of functional dyspepsia, IBS, gastric ulcer, lactose intolerance and reflux was equally distributed between groups. Each symptom item estimated by the VAS-IBS questionnaire was present in about half of all patients examined. Only 4 patients in each group did not have any form of gastrointestinal symptoms (Table 2). There was a wide variety in symptom intensity within each group also. None of the items in VAS-IBS correlated with age (data not shown).

Dietary patterns

All the participants who completed the nutrition questionnaire ($n = 42$) started the day with a breakfast, which in the vast majority of cases consisted of coffee or tea, together with bread and/or muesli and milk products. Twenty-seven participants had homemade lunch, whereas ten participants had lunch at a restaurant or had precooked meals, and five participants never had any lunch. Thirty-three participants had dinner at home, whereas eight participants had regular dinner at a restaurant or did not have dinner. Those who had homemade lunch suffered from more gastrointestinal symptoms compared with those who did not eat lunch, had lunch at a restaurant or had precooked meals, although bloating and flatulence was the only item that reached statistical significance [52 (25-93) *vs* 88 (70-100), $P = 0.024$]. The difference could not be related to any differences in socioeconomic factors or smoking or alcohol habits (data not shown) or in age span [66 (50-76) *vs* 65 (59-72), $P = 0.851$]. When the patients were divided into three groups depending on lunch habits [(1) home-maid lunch; (2) lunch at a restaurant or precooked meals; and (3) no lunch], those who had homemade lunch registered the most severe gastrointestinal symptoms on all the VAS scales, although the differences did not reach statistical significance (data not shown).

Microbiota and inflammatory biomarkers

Patients with DD had significantly higher levels of *Enterobacteriaceae* than patients without diverticula ($P = 0.043$; Table 3). Although patients with DD more

Table 1 Basal characteristics of the subjects *n* (%)

Characteristic	Diverticular disease, *n* = 16	No diverticula, *n* = 35	*P* value
Age in year	68 (62-76)	62 (40-74)	0.072
Sex, male/female	6/10	18/17	0.384
Body mass index in kg/m^2	27 (24-30)	25 (22-27)	0.136
Education			0.001
Primary school	9 (60.0)	7 (20.0)	
Secondary school	1 (6.7)	20 (57.1)	
Higher education	4 (26.7)	6 (17.1)	
Missing	1 (6.7)	2 (5.7)	
Occupation			0.332
Working/studying	4 (25.0)	15 (42.9)	
Retired	9 (56.3)	16 (45.7)	
Sick leave/disability	2 (12.5)	2 (5.7)	
Missing	1 (6.3)	2 (5.7)	
Civil status			0.376
Single/living alone	2 (12.5)	2 (5.7)	
Married/cohabitation	8 (50.0)	22 (62.9)	
Divorced/widowed	5 (31.3)	6 (17.1)	
Missing	1 (6.3)	5 (14.3)	
Physical activity			0.033
Mostly sitting	5 (31.3)	1 (2.9)	
Light activity	6 (37.5)	15 (42.9)	
Moderate but regular activity	3 (18.8)	14 (40.0)	
Regular activity	1 (6.3)	3 (8.6)	
Missing	1 (6.3)	2 (5.7)	
Smoking			0.668
Never smoked	4 (25.0)	13 (37.1)	
Former smokers	7 (43.8)	15 (42.9)	
Current smokers	4 (25.1)	5 (14.3)	
Missing	1 (6.3)	2 (5.7)	
Alcohol intake frequency			0.765
Never	4 (25.0)	4 (11.4)	
Once monthly or less	3 (18.8)	10 (28.6)	
2-4 times a month	3 (18.8)	9 (25.7)	
2-3 times a week	4 (25.0)	8 (22.9)	
⩾ 4 times a week	1 (6.3)	2 (5.7)	
Missing	1 (6.3)	2 (5.7)	
Alcohol amount at each intake			0.231
1-2 glasses	7 (43.8)	20 (57.1)	
3-4 glasses	2 (12.5)	7 (20.0)	
⩾ 5 glasses	2 (12.5)	1 (2.9)	
Missing	5 (31.3)	6 (17.2)	
Alcohol intake of 6 or more glasses			0.361
Never	9 (56.3)	15 (42.9)	
Once monthly or less	5 (31.3)	11 (31.4)	
Daily or several days a week	1 (6.3)	4 (11.4)	
Missing	1 (6.3)	5 (14.3)	
Antibiotic use last 6 mo	5 (31.3)	5 (14.3)	0.299
Probiotic use	2 (1.3)	2 (6.1)	0.701
Vaginal delivery	15 (93.8)	31 (88.6)	1

Values are presented as median (interquartile ranges), unless otherwise indicated. Differences between groups were calculated by Fisher's exact test or Mann-Whitney *U* test. $P < 0.05$ was considered statistically significant.

often had lower education and less physical activity, the different subgroups of these parameters did not affect the amount of *Enterobacteriaceae,* diversity indices of Shannon-Wiener or Simpson, or the number of T-RFs ($P = 0.413$, $P = 0.803$, $P = 0.770$, and $P = 0.588$, respectively, *vs* $P = 0.684$, $P = 0.616$, $P = 0.745$, and $P = 0.316$, respectively). There were no differences in any parameters between controls with and without polyps (data not shown).

There was an inverse correlation between the amount of *Enterobacteriaceae* and Simpson's index (rs = -0.361, $P = 0.033$) and a tendency to correlation between *Enterobacteriaceae* and Shannon-Wiener index (rs = -0.299, $P = 0.081$). The Shannon-Wiener and Simpson's indices correlated with each other (rs = 0.947, $P < 0.001$) and number of T-RFs (rs = 0.917, $P < 0.001$ and rs = 0.772, $P < 0.001$, respectively).

Several of the patients had humoral inflammatory parameters above or beneath the reference values, *i.e.*, plasma-C-reactive protein (CRP): < 3 mg/L; blood-

Table 2 Legree of symptoms based on Visual Analog Scale for Irritable Bowel

	Diverticular disease, *n* = 16	No diverticula, *n* = 35	*P* value	Symptom level, median	Symptom, *n* (%)	*P* value
VAS-IBS, median (IQR)						
Abdominal pain	81 (49-100)	84 (48-100)	0.759	95	21 (60)/9 (56)	1
Diarrhea	96 (61-100)	83 (50-100)	0.404	97	23 (66)/8 (50)	0.506
Constipation	95 (52-100)	98 (54-100)	0.613	91	14 (40)/7 (44)	1
Bloating and flatulence	75 (23-100)	61 (40-100)	0.711	85	22 (63)/9 (56)	0.749
Nausea and vomiting	93 (48-100)	97 (80-100)	0.347	98	17 (49)/9 (56)	0.756
Absence of any GI symptom	4 (25)	4 (11.4)	0.236			
GI comorbidities, *n* (%)						
Celiac disease	0	0				
Functional dyspepsia	5 (31.3)	8 (22.9)	0.509			
IBS	5 (31.3)	17 (48.6)	0.212			
Gastric ulcer	5 (31.3)	7 (20.0)	0.476			
Lactose intolerance	1 (6.3)	0	0.093			
Reflux	5 (31.3)	9 (25.7)	0.738			

Data are presented as median [interquartile range (IQR)] or number and percentages. Symptom number is the number in each group presenting with symptoms. The level of VAS-IBS used to differentiate between symptoms or not is defined as a score above the median values in healthy subjects (No 22). Mann-Whitney *U* test or Fisher's exact test. *P*-value < 0.05 was considered statistically significant. GI: Gastrointestinal; IBS: Irritable bowel syndrome; VAS-IBS: Visual Analog Scale for Irritable Bowel Syndrome.

Table 3 Mucosal count of Enterobacteriaceae and gut microbiota diversity and humoral inflammatory biomarkers

	Diverticular disease, *n* = 16	No diverticula, *n* = 35	*P* value
Enterobacteriaceae, log10 16S rRNA genes/g	9.27 (7.34-10.04)	7.76 (7.13-8.76)	0.043
Shannon-Wiener index	2.02 (1.80-2.36)	2.30 (1.94-2.48)	0.401
Simpson's index	0.80 (0.75-0.86)	0.82 (0.76-0.88)	0.383
T-RF, *n*	17.0 (11.0-21.0)	17.0 (12.5-22.0)	0.776
P-CRP, mg/L	4.40 (1.38-5.80)	1.70 (0.60-6.00)	0.346
B-leukocytes, 10^9/L	8.40 (6.38-9.98)	8.10 (5.90-8.85)	0.466
B-thrombocytes, 10^9/L	289 (219-334)	219 (186-266)	0.149
P-albumin, g/L	36 (34-42)	36 (34-40)	0.819

Gut microbiota was analyzed in feces, and inflammatory biomarkers in blood or plasma. Data are presented as median (interquartile range). Mann-Whitney *U* test. *P*-value < 0.05 was considered statistically significant. B: Blood; P: Plasma; T-RF: Terminal restriction fragments.

leucocytes: 3.5-8.8 × 10^9/L; blood-thrombocytes 125-340 × 10^9/L; and plasma-albumin: 36-48 g/L. The level of inflammatory biomarkers did not differ between patients with or without DD (Table 3). Neither did presence nor absence of IBS affect the plasma levels of CRP (P = 0.194) and albumin (P = 0.902), or blood levels of leukocytes (P = 0.912) and thrombocytes (P = 0.509). There was no correlation between any of the inflammatory biomarkers and the level of *Enterobacteriaceae* or bacterial diversity (data not shown).

Neither the amount of *Enterobacteriaceae* nor the diversity indices correlated with age, BMI, or any items of the VAS-IBS (data not shown). When calculating differences between patients with and without any of the gastrointestinal symptoms, there were no differences in amount of *Enterobacteriaceae* or diversity indices (data not shown). Presence of IBS did not affect the amount of *Enterobacteriaceae* (P = 0.867), Shannon-Wiener index (P = 0.533), Simpson's index (P = 0.478), or number of T-RFs (P = 0.828).

There were no differences in the amount of *Enterobacteriaceae* or the diversity indices between those who had a regular *vs* irregular breakfast intake of coffee/tea, dairy products, or cereals. The gut microbiota parameters examined were not influenced by intake of homemade lunch or dinner, smoking and alcohol habits, intake of probiotics and antibiotics, or movement patterns (data not shown).

DISCUSSION

In the present study examining symptomatic patients with elective colonoscopy, patients with DD had higher amount of *Enterobacteriaceae* compared with patients without diverticula, whereas the presence of gastrointestinal symptoms or IBS did not affect the amount of *Enterobacteriaceae.* Patients who had homemade lunch showed more symptoms of bloating and flatulence than those who did not have any lunch or had lunch at a restaurant/precooked meal. None of the studied lifestyle and socioeconomic parameters affected the amount of *Enterobacteriaceae* or bacterial diversity of the gut.

The present result of higher levels of *Enterobacteriaceae* in mucosa of DD is in opposite to the previous result of Barbara *et al*[17]. The differences may be explained by the different study design and different composition of the control group. The present study enrolled mainly symptomatic patients examined by colonoscopy to exclude organic diseases or patients with heredity for colon cancer. Barbara *et al*[17] used asymptomatic or symptomatic patients enrolled to colonoscopy in a screening program to exclude malignancy or as follow-up after polyp resections. Thus, the control group in Barbara *et al*[17] consisted of a smaller cohort (n = 14) of asymptomatic subjects, and a lower percentage of symptomatic DD, with sex and age differences between groups. The microbiota composition differed between mucosal biopsies and feces[17]. We decided not to analyze fecal microbiota in our study, since there are greater differences between fecal and mucosal microbiota than between individual subjects, and it is considered more reliable to measure microbiota composition in mucosa than feces[29]. The general composition estimated by microbial diversity may be more important to health than the levels of individual bacterial strains[9,10,14].

Abundance of *Enterobacteriaceae/Escherichia coli* is associated with IBD, both in animal models and in humans[12,13,15,16]. The gut microbiota generates biologically active small molecules, *e.g*., amino acids, short-chain fatty acids, sugars and organic acids, which are presumed to affect the health of the host[30]. Basic microbiome metabolism was altered in IBD, with reduced amino acid synthesis and carbohydrate metabolism and increased nutrient uptake. Furthermore, genes involved in pathogenesis processes such as secretion of enterotoxins, wall-degrading enzymes and cytokine production were over-represented in Crohn's disease[13]. This would lead to tissue destruction and bacterial overgrowth, with structural and functional dysbiosis.

In the present study of DD, the abundance of *Enterobacteriaceae* in the colon mucosa at a distance from the diverticula could hypothetically reflect a low-grade inflammation in the bowel wall. The previous publication by Barabra *et al*[17] suggested chronic low-grade gut mucosa inflammation in DD, through histopathological examination. Such low-grade inflammation was not reflected in the humoral inflammatory system, confirmed by overall normal CRP and blood cells levels, but may be captured in mucosal biopsies[17,31]. A low-grade inflammation may contribute to pain sensitization and visceral hypersensitivity and symptom development[7,8], which contributes to the increased risk of IBS after acute diverticulitis[6].

It remains unclear whether microbial changes are a cause or a consequence of DD. We do not know whether inflammation is a primary event, leading to weakening of the bowel wall and eventually to development of diverticula, or if inflammation is secondary to the presence of DD distant in the bowel with retention of luminal contents and bacterial overgrowth. Even if the microbial changes are secondary, the dysbiosis may further accelerate the pathologic process and weakening of the bowel wall by mechanisms explained above[13].

Microbial dysbiosis in combination with genetic, environmental, and psychosocial factors are proposed to be involved in the etiology of IBS[20,32]. *Escherichia coli* was increased in Chinese IBS patients compared with controls, whereas no differences of these feces bacteria were found between IBS patients and healthy controls from other regions[33]. This is in line with our present study, which did not show any correlations between gastrointestinal symptoms or IBS and *Enterobacteriaceae.*

Gastrointestinal symptoms without visible organic damages are called functional bowel disorders, where IBS is the most common of the disorders, with a prevalence of 10%-15% in the population[20]. A great deal of the present patients suffered from IBS or IBS-like symptoms, whereas some patients experienced gastrointestinal symptoms without fulfilling the Rome IV criteria[20]. Symptomatology is not enough to distinguish between different bowel disorders, as found in the present study. It has previously been shown that patients with IBS have as severe symptoms as those with organic changes, *i.e.,* primary Sjögren´s syndrome and enteric dysmotility[34]. A great symptomatic overlap between DD and IBS is described previously[1], which further underlines that disease classification must be based on organic criteria and not on symptoms solely. Biomarkers for IBS and DD are lacking, but measurements of markers of dysbiosis, inflammatory cells in mucosa, and metabolomes may be able to distinguish IBS from DD in the future. Probiotic therapy is an efficient treatment of IBS[35], whereas the evidence of efficiency in treatment of DD is insufficient.

Since this was a cross-sectional study, we do not know the reason for more symptoms being present in the group with homemade food. The reason may depend on patients with more severe symptoms avoiding visiting a restaurant, to have better control over their food intake.

The strength in the present pilot study is that we have analyzed mucosal biopsies instead of feces. The mucosa microbiota composition is anticipated to be more reliable than the feces composition. To compare another patient group with similar degree of symptoms seems more appropriate than to compare DD with healthy, non-symptomatic subjects. Further, we have considered food intake and other lifestyle habits affecting microbiota composition. The weakness is the small cohort size. Furthermore, since the patients were enrolled consecutively, there was no matching between cases and controls of, *e.g*., age, sex or lifestyle habits. In a larger study, some of the demographic parameters and lifestyle habits could have shown statistically significant influence on the gut microbiota. We chose to initially perform this as a pilot trial with a limited amount of patients, as the methodology is very expensive. Since it now has been shown that there are differences

in DD according to the gut microbiota, it is important to continue with further studies and more extensive analyses. Since this was a cross-sectional study, we do not know whether the microbial alterations are primary in the development of diverticula or just secondary to DD, with retention of luminal content.

In this pilot study, patients with DD had higher amount of *Enterobacteriaceae* in the colon mucosa compared to patients without DD. Assessment of gut microbiota may distinguish DD from other patient groups and may be involved in etiology and pathophysiology of the disease. Gastrointestinal symptomatology seems to not be related to the amount of *Enterobacteriaceae* or to the bacterial diversity.

ARTICLE HIGHLIGHTS

Research background

Diverticular disease (DD) is a common gastrointestinal disease of unknown etiology. The symptoms of DD are similar with symptoms of irritable bowel syndrome (IBS). The gut microbiota is discussed as important for the etiology and pathophysiology in a wide range of diseases. Bacterial diversity is higher in lean compared to obese individuals, and in healthy states compared to unhealthy states, and some bacterial groups, *e.g.*, *Enterobacteriaceae,* are associated with over-weight and inflammation. The family *Enterobacteriaceae* is commonly found in the gut ecosystem, where *Escherichia coli* is the most abundant species of the family. Only a few studies have been performed regarding microbial composition in DD. Recently, lower amounts of *Enterobacteriaceae* were found in the colon mucosa of DD patients compared with healthy controls, whereas higher amounts of *Akkermansia* and no difference in the *Escherichia coli* subgroup were found in feces in another DD cohort. Thus, it is hypothesized that gut microbiota is involved in the etiology and pathophysiology of DD, but the few studies performed so far have shown inconclusive results.

Research motivation

Today, there is no efficient treatment option for DD, neither to prevent disease development nor to reduce the symptoms when the disease has been established, which renders a lot of suffering to the patients. To find out the etiology is crucial to be able to prevent and efficiently treat the disease. New knowledge within this disease field may point out the direction for future research.

Research objectives

The primary aim of the present study was to compare the level of the large Gram-negative bacterial family *Enterobacteriaceae* and gut bacterial diversity in colon mucosa between consecutive patients diagnosed with DD and patients with normal endoscopic findings. Secondary aims were to evaluate the influence of demography, socioeconomic status, lifestyle habits, inflammatory parameters and gastrointestinal symptoms on the gut microbiota. These objectives were possible to realize by the present study design. Further studies according to the same study design, but with larger patient cohorts, are important to perform to confirm the results.

Research methods

All consecutive patients referred to elective colonoscopy at the Department of Endoscopy, Skåne University Hospital, Malmö, were invited to participate in the study. If the patients agreed to participate, they had to complete a study questionnaire about demography, socioeconomic status, lifestyle habits, family history and medical history, the Visual Analog Scale for Irritable Bowel Syndrome (VAS-IBS), and a nutrition questionnaire to analyze dietary habits. The colonoscopy was performed according to clinical routines. Four different mucosa biopsies were obtained from the mid part of the colon descendens. Samples were stored at -80 °C until the gut microbiota was analyzed by quantitative polymerase chain reaction (qPCR) and terminal restriction fragment length polymorphism (T-RFLP). Blood samples were collected according to clinical routines and analyzed at the Department of Clinical Chemistry. A study protocol was completed by the physician about clinical findings and histopathological diagnoses. The patients were divided into two groups depending on the colonoscopy finding: patients with DD, and patients without any diverticula who served as controls. Three of the four mucosa samples, mean weight 15 ± 0.6 mg, were used for DNA extraction. DNA was isolated and purified by EZ1 Advanced XL (EZ1 DNA Tissue Kit and Bacteria Card; Qiagen, Hilden, Germany). The amount of *Enterobacteriaceae* was estimated using a qPCR assay according to Karlsson *et al*. Primers used for the qPCR assay have been used and published previously. The detection limit was 10^2 genes/reaction. For standard curves, 10-fold dilution series of the target DNA were made in EB buffer (Qiagen). Number of bacteria was expressed as $\log_{10}$ 16S rRNA genes/g feces. T-RFLP was applied to assess the microbial diversity, as previously described. Thresholds for internal standard and terminal restriction fragments (T-RFs) were set to 5 and 15 fluorescence units, respectively. Microbial diversity was estimated by calculation of richness (number of T-RFs) and Shannon-Wiener and Simpson's diversity indices as described by Karlsson *et al*, with the exception that T-RFs within 40-580 base pairs were included in the T-RFLP profile analysis and calculation. The diversity indices take into account both richness and evenness when considering the relative abundance of bacterial groups. Both indices are commonly used to assess microbial diversity. Samples below the limit of detection (in qPCR) were replaced by the limit of detection for statistical analysis.

Research results

Finally, 51 patients were included in the present study, 16 with DD and 35 controls without organic changes visible at the colonoscopy or at the histopathological examination (n = 12), except non-malignant polyps (n = 23). The reasons for referral to colonoscopy were presence of gastrointestinal symptoms which rendered a colonoscopy to exclude inflammatory bowel disease, malignancy or DD (n = 17), follow-up after previous resection of polyps (n = 17), rectal bleeding (n = 11), screening for cancer due to heredity (n = 4), or perforation to the urinary tract (n = 2). Only one subject in the DD group had a history of verified acute diverticulitis. There was an equal sex distribution in the groups. Subjects without DD were slightly older than controls [68 (62-76) years *vs* 62 (40-74) years, P = 0.072]. Altogether, 22 patients (43.1%) fulfilled the Rome IV criteria for IBS. The prevalence of functional dyspepsia, IBS, gastric ulcer, lactose intolerance and reflux was equally distributed between groups. Each symptom item estimated by the VAS-IBS questionnaire was present in about half of all patients examined. Only 4 patients in each group did not have any form of gastrointestinal symptoms. There was a wide variety in symptom intensity within each group also. None of the items in VAS-IBS correlated with age. Those who had homemade lunch suffered from more gastrointestinal symptoms compared with those who did not eat lunch, had lunch at a restaurant or had precooked meals, although bloating and flatulence was the only item that reached statistical significance [52 (25-93) *vs* 88 (70-100), P = 0.024]. The difference could not be related to any differences in socioeconomic factors or smoking or alcohol habits or in age span [66 (50-76) *vs* 65 (59-72), P = 0.851]. Patients with DD had significantly higher levels of *Enterobacteriaceae* than patients without diverticula (P = 0.043). Although patients with DD more often had lower education and less physical activity, the different subgroups of these parameters did not affect the amount of *Enterobacteriaceae,* diversity indices of Shannon-Wiener or Simpson, or the number of T-RFs (P = 0.413, P = 0.803, P = 0.770, and P = 0.588, respectively, *vs* P = 0.684, P = 0.616, P = 0.745, and P = 0.316, respectively). There were no differences in any parameters between controls with and without polyps. There was an inverse correlation between the amount of *Enterobacteriaceae* and Simpson's index (rs = -0.361, P = 0.033) and a tendency to correlation between *Enterobacteriaceae* and Shannon-Wiener index (rs = -0.299, P = 0.081). The Shannon-Wiener and Simpson's indices correlated with each other (rs = 0.947, P < 0.001) and number of T-RFs (rs = 0.917, P < 0.001 and rs = 0.772, P < 0.001, respectively). Several of the patients had humoral inflammatory parameters above or beneath the reference values, *i.e.* plasma-C-reactive protein (CRP): < 3 mg/L; blood-leucocytes: 3.5-8.8 × 10^9/L; blood-thrombocytes 125-340 × 10^9/L; and plasma-albumin: 36-48 g/L. The level of inflammatory biomarkers did not differ between patients with or without DD. Neither did presence nor absence of IBS affect the plasma levels of CRP (P = 0.194) and albumin (P = 0.902), or blood levels of leukocytes (P = 0.912) and thrombocytes (P = 0.509). There was no correlation between any of the inflammatory biomarkers and the level of *Enterobacteriaceae* or bacterial

diversity. Neither the amount of *Enterobacteriaceae* nor the diversity indices correlated with age, BMI, or any items of the VAS-IBS. When calculating differences between patients with and without any of the gastrointestinal symptoms, there were no differences in amount of *Enterobacteriaceae* or diversity indices (data not shown). Presence of IBS did not affect the amount of *Enterobacteriaceae* (P = 0.867), Shannon-Wiener index (P = 0.533), Simpson' s index (P = 0.478), or number of T-RFs (P = 0.828). There were no differences in the amount of *Enterobacteriaceae* or the diversity indices between those who had a regular *vs* irregular breakfast intake of coffee/tea, dairy products, or cereals. The gut microbiota parameters examined were not influenced by intake of homemade lunch or dinner, smoking and alcohol habits, intake of probiotics and antibiotics, or movement patterns. The problems that remain to be solved are whether the difference in gut microbiota composition are primary events in the disease development or secondary to the DD. The causality to DD must still be defined.

Research conclusions

The new finding of the present study is the abundance of *Enterobacteriaceae* in colon mucosa in DD, and that this abundance was not related to age, BMI, socioeconomic parameters, gastrointestinal symptoms or lifestyle habits. Microbial diversity was not affected by DD or any other parameters measured. The new theory that this study proposes is that the composition of gut microbiota is involved in DD. The summarization of this study is that gut microbiota may be affected in patients with DD. This study is the first study where a clinical cohort of patients is consecutively enrolled during colonoscopy to analyze gut microbiota in colon mucosa, where the only difference between the groups compared is the presence or absence of colon diverticula. Previous studies have enrolled participants in screening programs or analyzed microbiota composition in feces. The authors also studied socioeconomic features and lifestyle habits in the cohort, to be able to adjust for confounders. The new hypotheses proposed are that gut microbiota is involved in DD and that demography, socioeconomic parameters and dietary habits may be of less importance for the microbiota than the presence or absence of colon diverticula. The new methods proposed are the enrolment of consecutive clinical patients in scientific trials, analyses of gut microbiota in mucosa instead of feces, analysis of microbial diversity to get a general reflection of the gut microbiota, analysis of the amount of *Enterobacteriaceae* or other bacteria by qPCR, and estimation of gastrointestinal symptoms by the VAS-IBS questionnaire. The new phenomenon found were that presence or absence of colon diverticula are more important for gut microbiota than demography, socioeconomic parameters, gastrointestinal symptoms, or lifestyle habits. Another new phenomenon was that patients with homemade lunch had more gastrointestinal symptoms than patients who did not eat lunch or had lunch at a restaurant. The authors confirmed the hypothesis that the amount of *Enterobacteriaceae* was affected by DD, but failed to confirm the hypothesis that overall bacterial diversity was influenced by colon diverticula. The authors also failed to confirm the hypotheses that demography, socioeconomic parameters, gastrointestinal symptoms and lifestyle habits were associated with gut microbiota composition. The major implication for clinical practice in the future is to consider dysbiosis in patients with DD. Tests to determine gut microbiota are available for clinical use, and should be considered in the management of these patients.

Research perspectives

The experience the authors have learnt from this study is that presence or absence of DD is more important for the gut microbiota composition than demography, socioeconomic parameters, gastrointestinal symptoms, and lifestyle habits. The authors have also learnt from this study that homemade food is not always the best for patients in the management of gastrointestinal symptoms. The authors must further study the importance of gut microbiota in DD. The authors should continue to include patients with DD in experiments to analyze gut microbiota composition to get larger cohorts, and to perform clinical trials to evaluate the effect of probiotics in symptom management of DD. The best method is to analyze gut microbiota in colon mucosa instead of feces. The VAS-IBS is also a useful tool to estimate gastrointestinal symptoms.

REFERENCES

1 **Cuomo R**, Barbara G, Andreozzi P, Bassotti G, Casetti T, Grassini M, Ierardi E, Maconi G, Marchi S, Sarnelli G, Savarino V, Usai P, Vozzella L, Annibale B. Symptom patterns can distinguish diverticular disease from irritable bowel syndrome. *Eur J Clin Invest* 2013; **43**: 1147-1155 [PMID: 23992370 DOI: 10.1111/eci.12152]

2 **Stollman N**, Raskin JB. Diverticular disease of the colon. *Lancet* 2004; **363**: 631-639 [PMID: 14987890 DOI: 10.1016/S0140-6736(04)15597-9]

3 **Hjern F**, Johansson C, Mellgren A, Baxter NN, Hjern A. Diverticular disease and migration--the influence of acculturation to a Western lifestyle on diverticular disease. *Aliment Pharmacol Ther* 2006; **23**: 797-805 [PMID: 16556182 DOI: 10.1111/j.1365-2036.2006.02805.x]

4 **Shahedi K**, Fuller G, Bolus R, Cohen E, Vu M, Shah R, Agarwal N, Kaneshiro M, Atia M, Sheen V, Kurzbard N, van Oijen MG, Yen L, Hodgkins P, Erder MH, Spiegel B. Long-term risk of acute diverticulitis among patients with incidental diverticulosis found during colonoscopy. *Clin Gastroenterol Hepatol* 2013; **11**: 1609-1613 [PMID: 23856358 DOI: 10.1016/j.cgh.2013.06.020]

5 **Humes DJ**, Simpson J, Neal KR, Scholefield JH, Spiller RC. Psychological and colonic factors in painful diverticulosis. *Br J Surg* 2008; **95**: 195-198 [PMID: 17939130 DOI: 10.1002/bjs.5962]

6 **Cohen E**, Fuller G, Bolus R, Modi R, Vu M, Shahedi K, Shah R, Atia M, Kurzbard N, Sheen V, Agarwal N, Kaneshiro M, Yen L, Hodgkins P, Erder MH, Spiegel B. Increased risk for irritable bowel syndrome after acute diverticulitis. *Clin Gastroenterol Hepatol* 2013; **11**: 1614-1619 [PMID: 23524129 DOI: 10.1016/j.cgh.2013.03.007]

7 **Simpson J**, Scholefield JH, Spiller RC. Origin of symptoms in diverticular disease. *Br J Surg* 2003; **90**: 899-908 [PMID: 12905541 DOI: 10.1002/bjs.4277]

8 **Clemens CH**, Samsom M, Roelofs J, van Berge Henegouwen GP, Smout AJ. Colorectal visceral perception in diverticular disease. *Gut* 2004; **53**: 717-722 [PMID: 15082591 DOI: 10.1136/gut.2003.018093]

9 **Hakansson A**, Molin G. Gut microbiota and inflammation. *Nutrients* 2011; **3**: 637-682 [PMID: 22254115 DOI: 10.3390/nu3060637]

10 **Karlsson CL**, Onnerfält J, Xu J, Molin G, Ahrné S, Thorngren-Jerneck K. The microbiota of the gut in preschool children with normal and excessive body weight. *Obesity* (Silver Spring) 2012; **20**: 2257-2261 [PMID: 22546742 DOI: 10.1038/oby.2012.110]

11 **Fåk F**, Karlsson CL, Ahrné S, Molin G, Weström B. Effects of a high-fat diet during pregnancy and lactation are modulated by E. coli in rat offspring. *Int J Obes* (Lond) 2012; **36**: 744-751 [PMID: 21730967 DOI: 10.1038/ijo.2011.118]

12 **Baumgart M**, Dogan B, Rishniw M, Weitzman G, Bosworth B, Yantiss R, Orsi RH, Wiedmann M, McDonough P, Kim SG, Berg D, Schukken Y, Scherl E, Simpson KW. Culture independent analysis of ileal mucosa reveals a selective increase in invasive Escherichia coli of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. *ISME J* 2007; **1**: 403-418 [PMID: 18043660 DOI: 10.1038/ismej.2007.52]

13 **Morgan XC**, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, Reyes JA, Shah SA, LeLeiko N, Snapper SB, Bousvaros A, Korzenik J, Sands BE, Xavier RJ, Huttenhower C. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol* 2012; **13**: R79 [PMID: 23013615 DOI: 10.1186/gb-2012-13-9-r79]

14 **Mondot S**, Lepage P. The human gut microbiome and its dysfunctions through the meta-omics prism. *Ann N Y Acad Sci* 2016; **1372**: 9-19 [PMID: 26945826 DOI: 10.1111/nyas.13033]

15 **Arthur JC**, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan TJ, Campbell BJ, Abujamel T, Dogan B, Rogers AB, Rhodes JM, Stintzi A, Simpson KW, Hansen JJ, Keku TO, Fodor AA, Jobin C. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 2012; **338**: 120-123 [PMID: 22903521 DOI: 10.1126/science.1224820]

16 **Garrett WS**, Gallini CA, Yatsunenko T, Michaud M, DuBois A, Delaney ML, Punit S, Karlsson M, Bry L, Glickman JN, Gordon JI, Onderdonk AB, Glimcher LH. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally

transmitted colitis. *Cell Host Microbe* 2010; **8**: 292-300 [PMID: 20833380 DOI: 10.1016/j.chom.2010.08.004]
17 **Barbara G**, Scaioli E, Barbaro MR, Biagi E, Laghi L, Cremon C, Marasco G, Colecchia A, Picone G, Salfi N, Capozzi F, Brigidi P, Festi D. Gut microbiota, metabolome and immune signatures in patients with uncomplicated diverticular disease. *Gut* 2017; **66**: 1252-1261 [PMID: 27618836 DOI: 10.1136/gutjnl-2016-312377]
18 **Tursi A**, Mastromarino P, Capobianco D, Elisei W, Miccheli A, Capuani G, Tomassini A, Campagna G, Picchio M, Giorgetti G, Fabiocchi F, Brandimarte G. Assessment of Fecal Microbiota and Fecal Metabolome in Symptomatic Uncomplicated Diverticular Disease of the Colon. *J Clin Gastroenterol* 2016; **50** Suppl 1: S9-S12 [PMID: 27622378 DOI: 10.1097/MCG.0000000000000626]
19 **Stanghellini V**, Chan FK, Hasler WL, Malagelada JR, Suzuki H, Tack J, Talley NJ. Gastroduodenal Disorders. *Gastroenterology* 2016; **150**: 1380-1392 [PMID: 27147122 DOI: 10.1053/j.gastro.2016.02.011]
20 **Mearin F**, Lacy BE, Chang L, Chey WD, Lembo AJ, Simren M, Spiller R. Bowel Disorders. *Gastroenterology* 2016 [PMID: 27144627 DOI: 10.1053/j.gastro.2016.02.031]
21 **Bengtsson M**, Ohlsson B, Ulander K. Development and psychometric testing of the Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS). *BMC Gastroenterol* 2007; **7**: 16 [PMID: 17475020 DOI: 10.1186/1471-230X-7-16]
22 **Bengtsson M**, Persson J, Sjölund K, Ohlsson B. Further validation of the visual analogue scale for irritable bowel syndrome after use in clinical practice. *Gastroenterol Nurs* 2013; **36**: 188-198 [PMID: 23732784 DOI: 10.1097/SGA.0b013e3182945881]
23 **Stenblom EL**, Weström B, Linninge C, Bonn P, Farrell M, Rehfeld JF, Montelius C. Dietary green-plant thylakoids decrease gastric emptying and gut transit, promote changes in the gut microbial flora, but does not cause steatorrhea. *Nutr Metab* (Lond) 2016; **13**: 67 [PMID: 27777602 DOI: 10.1186/s12986-016-0128-4]
24 **Bartosch S**, Fite A, Macfarlane GT, McMurdo ME. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Appl Environ Microbiol* 2004; **70**: 3575-3581 [PMID: 15184159 DOI: 10.1128/AEM.70.6.3575-3581.2004]
25 **Castillo M**, Martín-Orúe SM, Manzanilla EG, Badiola I, Martín M, Gasa J. Quantification of total bacteria, enterobacteria and lactobacilli populations in pig digesta by real-time PCR. *Vet Microbiol* 2006; **114**: 165-170 [PMID: 16384658 DOI: 10.1016/j.vetmic.2005.11.055]
26 **Sand E**, Linninge C, Lozinska L, Egecioglu E, Roth B, Molin G, Weström B, Ekblad E, Ohlsson B. Buserelin treatment to rats causes enteric neurodegeneration with moderate effects on CRF-immunoreactive neurons and Enterobacteriaceae in colon, and in acetylcholine-mediated permeability in ileum. *BMC Res Notes* 2015; **8**: 824 [PMID: 26710832 DOI: 10.1186/s13104-015-1800-x]
27 **Magurran AE.** An index of diversity. In measuring biological diversity. Oxford: Blackwell Science Ltd, 2004
28 **Walsh AJ**, Bryant RV, Travis SP. Current best practice for disease activity assessment in IBD. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 567-579 [PMID: 27580684 DOI: 10.1038/nrgastro.2016.128]
29 **Tang MS**, Poles J, Leung JM, Wolff MJ, Davenport M, Lee SC, Lim YA, Chua KH, Loke P, Cho I. Inferred metagenomic comparison of mucosal and fecal microbiota from individuals undergoing routine screening colonoscopy reveals similar differences observed during active inflammation. *Gut Microbes* 2015; **6**: 48-56 [PMID: 25559083 DOI: 10.1080/19490976.2014.1000080]
30 **Martinez KB**, Leone V, Chang EB. Microbial metabolites in health and disease: Navigating the unknown in search of function. *J Biol Chem* 2017; **292**: 8553-8559 [PMID: 28389566 DOI: 10.1074/jbc.R116.752899]
31 **Humes DJ**, Simpson J, Smith J, Sutton P, Zaitoun A, Bush D, Bennett A, Scholefield JH, Spiller RC. Visceral hypersensitivity in symptomatic diverticular disease and the role of neuropeptides and low grade inflammation. *Neurogastroenterol Motil* 2012; **24**: 318-e163 [PMID: 22276853 DOI: 10.1111/j.1365-2982.2011.01863.x]
32 **Distrutti E**, Monaldi L, Ricci P, Fiorucci S. Gut microbiota role in irritable bowel syndrome: New therapeutic strategies. *World J Gastroenterol* 2016; **22**: 2219-2241 [PMID: 26900286 DOI: 10.3748/wjg.v22.i7.2219]
33 **Zhuang X**, Xiong L, Li L, Li M, Chen M. Alterations of gut microbiota in patients with irritable bowel syndrome: A systematic review and meta-analysis. *J Gastroenterol Hepatol* 2017; **32**: 28-38 [PMID: 27300149 DOI: 10.1111/jgh.13471]
34 **Bengtsson M**, Hammar O, Mandl T, Ohlsson B. Evaluation of gastrointestinal symptoms in different patient groups using the visual analogue scale for irritable bowel syndrome (VAS-IBS). *BMC Gastroenterol* 2011; **11**: 122 [PMID: 22073983 DOI: 10.1186/1471-2393-13-201]
35 **Ford AC**, Quigley EM, Lacy BE, Lembo AJ, Saito YA, Schiller LR, Soffer EE, Spiegel BM, Moayyedi P. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. *Am J Gastroenterol* 2014; **109**: 1547-1561; quiz 1546, 1562 [PMID: 25070051 DOI: 10.1038/ajg.2014.202]

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World J Gastrointest Pathophysiol 2018 February 15; 9(1): 28-36

DOI: 10.4291/wjgp.v9.i1.28

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Observational Study

Liver cirrhosis-effect on QT interval and cardiac autonomic nervous system activity

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Author contributions: Tsiompanidis E, Siakavellas SI, Tentolouris A, Eleftheriadou I, Chorepsima S, Manolakis A, Oikonomou K and Tentolouris N were involved in the design of the study, interpretation of results and writing of the manuscript; Tsiompanidis E, Siakavellas SI and Manolakis A participated in data acquisition; Tentolouris A, Eleftheriadou I, Chorepsima S and Tentolouris N conducted the statistical analysis; all authors read and approved the final manuscript.

Institutional review board statement: The experimental protocol was reviewed and approved by the Ethics Committee of Laiko General Hospital (Number of Permission: 2903/10-12-2014).

Informed consent statement: The study was approved by the ethics committee of Laiko General Hospital and written informed consent was obtained from all individuals before participation in the study.

Conflict-of-interest statement: There are no conflicts of interest related to this work.

Manuscript source: Unsolicited manuscript

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Received: August 6, 2017
Peer-review started: August 7, 2017
First decision: September 7, 2017
Revised: October 4, 2017
Accepted: October 30, 2017
Article in press: October 30, 2017
Published online: February 15, 2018

Abstract

AIM

To examine the impact of liver cirrhosis on QT interval and cardiac autonomic neuropathy (CAN).

METHODS

A total of 51 patients with cirrhosis and 51 controls were examined. Standard 12-lead electrocardiogram recordings were obtained and QT as well as corrected QT interval (QTc) and their dispersions (dQT, dQTc) were measured and calculated using a computer-based program. The diagnosis of CAN was based upon the battery of the tests proposed by Ewing and Clarke and the consensus statements of the American Diabetes Association. CAN was diagnosed when two out of the four classical Ewing tests were abnormal.

RESULTS

QT, QTc and their dispersions were significantly longer ($P < 0.01$) in patients with cirrhosis than in controls. No

significant differences in QT interval were found among the subgroups according to the etiology of cirrhosis. Multivariate regression analysis after controlling for age, gender and duration of cirrhosis demonstrated significant association between QT and presence of diabetes mellitus [standardized regression coefficient (beta) = 0.45, P = 0.02] and treatment with diuretics (beta = 0.55, P = 0.03), but not with the Child-Pugh score (P = 0.54). Prevalence of CAN was common (54.9%) among patients with cirrhosis and its severity was associated with the Child-Pugh score (r = 0.33, P = 0.02). Moreover, patients with decompensated cirrhosis had more severe CAN that those with compensated cirrhosis (P = 0.03). No significant association was found between severity of CAN and QT interval duration.

CONCLUSION

Patients with cirrhosis have QT prolongation. Treatment with diuretics is associated with longer QT. CAN is common in patients with cirrhosis and its severity is associated with severity of the disease.

Key words: QT interval; Cardiac autonomic neuropathy; Cirrhotic cardiomyopathy; Child-Pugh score; Model for end-stage liver disease score; Liver cirrhosis

Core tip: QT interval is significantly prolonged in patients with liver cirrhosis and its duration is associated with the use of diuretics but not with the severity of the disease. More than half of the patients with cirrhosis have cardiac autonomic neuropathy (CAN), while CAN severity is associated strongly with the severity of cirrhosis.

Tsiompanidis E, Siakavellas SI, Tentolouris A, Eleftheriadou I, Chorepsima S, Manolakis A, Oikonomou K, Tentolouris N. Liver cirrhosis-effect on QT interval and cardiac autonomic nervous system activity. *World J Gastrointest Pathophysiol* 2018; 9(1): 28-36 Available from: URL: http://www.wjgnet.com/2150-5330/full/v9/i1/28.htm DOI: http://dx.doi.org/10.4291/wjgp.v9.i1.28

INTRODUCTION

Cirrhosis can affect many organs and systems of the body including cardiovascular and autonomic nervous system (ANS)[1-3]. Among the cardiovascular manifestations often encountered in cirrhotic patients, most common are increased baseline cardiac output, attenuated systolic and diastolic function, blunted ventricular response to stimuli and electrophysiological abnormalities, comprising a group of phenomena, commonly referred to as "cirrhotic cardiomyopathy"[1-3].

As for the involvement of the ANS in the cirrhotic-related manifestations, it has been considered as being the result of toxic, metabolic and immunologic disturbances affecting both the sympathetic and parasympathetic constituents of ANS[3,4]. Due to the close interrelation of the two systems-cardiovascular and ANS-an abnormal ANS function in cirrhotic patients has been shown to be reflected in several cardiac- and vascular-related parameters such as QT interval prolongation, heart rate variability (HRV) and arterial pressure changes, all components of the so-called cardiac autonomic neuropathy (CAN)[5,6].

Previous data in patients with diabetes mellitus have shown that CAN is associated with prolongation of QT interval[7]. Both CAN, even subclinical, and QT prolongation have been associated with increased all-cause mortality in patients with diabetes[7-9]. Interestingly, some studies have shown that the prolongation of QT interval in patients with cirrhosis has been associated with the severity and progression of the disease and with poorer survival in cirrhotic patients[10-13]. On the other hand, in other studies, even though prolonged QT was associated with more severe liver dysfunction, this has not been translated to higher mortality[14,15]. Moreover, it is interesting that the prolonged QTc was improved in most patients after liver transplantation, although the extent and degree of improvement is variable, indicating a functional and reversible "nature" of such dysfunction[16]. Similarly, in some studies CAN has been associated with the severity of liver disease[6,17].

In the present cross-sectional study, we examined the association between QT interval-related parameters with presence and severity of cirrhosis. In addition, we examined the prevalence of CAN and its association with QT interval in patients with cirrhosis.

MATERIALS AND METHODS

Participants

A total of 102 participants were recruited, 51 cirrhotic patients followed consecutively at the outpatient clinic of our hospital and 51 age- and gender-matched healthy controls who were hospital staff and relatives of the patients with cirrhosis. The diagnosis of cirrhosis was established by liver biopsy in 25 subjects. In cases where biopsy was contraindicated (n = 26), the patients had clinical, biochemical and ultrasonographical findings of cirrhosis. The patients were further classified according to the Child-Pugh grading system as having decompensated (Child-Pugh score $\geqslant$ 7, n = 29) or compensated (Child-Pugh score < 7, n = 22) cirrhosis. In addition, the model for end-stage liver disease (MELD) score was measured and the histologic activity index was used to stage liver disease in patients who underwent a liver biopsy[18,19]. Diabetes mellitus was diagnosed using the American Diabetes Association criteria[20].

Criteria for exclusion from the study were as follows: (1) any electrolyte disturbance; (2) diseases which may affect ANS activity and QT interval duration such as coronary artery disease, heart failure, atrial fibrillation,

amyloidosis, hepatocellular carcinoma, episode of infection or gastrointestinal bleeding in the last two months prior to the study; (3) medications which affect ANS activity and QT interval duration like calcium channel blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, digitalis, tricyclic antidepressants, sympathomimetics and anticholinergics; and (4) patients with any degree of hepatic encephalopathy. Patients receiving propranolol or other beta blockers were included in the study after they had stopped the medication for at least ten days prior to examination. In order to minimize the risk of variceal bleeding due to discontinuation of propranolol, only patients at low risk, documented through esophago-gastro-duodenoscopy (varices with a diameter less than 5 mm and without signs of bleeding), were recruited.

The study was approved by the ethics committee of our hospital and informed consent was obtained from all participants according to the principles of the Declaration of Helsinki[21].

Procedures

Blood, urine sampling and all tests were carried out early in the morning after overnight fast of 8-10 h in a room of stable temperature (22 ℃-24 ℃). All individuals refrained from smoking or drinking coffee prior to the examination. Body weight and height was measured in light clothing and body mass index (BMI) was calculated. Blood pressure was measured in the sitting position three consecutive times with 1 min interval in between and the mean value of the second and third measurements was calculated and used in the analysis.

Blood was drawn for determination of hemoglobin (Hb), white blood cell count, platelet count and biochemical measurements. Biochemical determinations were made on an automatic analyzer. Glycosylated hemoglobin (HbA1c) was measured using HPLC. Plasma insulin (Biosure, Brussels, Belgium; coefficient of variation < 5%) was determined by radioimmunoassay. Insulin resistance was calculated by the homeostasis model assessment equation (HOMA-IR)[22].

Assessment of CAN

The diagnosis of CAN was based upon the battery of the tests proposed by Ewing and Clarke and the consensus statements of the American Diabetes Association[23,24]. The heart rate response to slow deep breathing (deep breathing test), the Valsalva maneuver and the assumption of upright position (lying-to-standing test) were assessed from electrocardiographic (ECG) recordings of RR intervals automatically using the computer-aided examination system VariaCardio TF4 (Medical Research, Leeds, United Kingdom). The change in systolic blood pressure upon standing, expressed as the difference between the mean of the last two values obtained in the supine position and the value obtained 60 s after standing up, were recorded. The first three tests were evaluated according to the published age-related heart rate tests, while orthostatic hypotension was diagnosed when a fall in systolic blood pressure $\geqslant$ 20 mmHg and/or a fall in diastolic blood pressure $\geqslant$ 10 mmHg were observed. Diagnosis of CAN was established when at least two out of four tests were abnormal[23,24]. In order to evaluate the severity of the CAN, each normal test was graded with 0.0, each borderline with 1.0 and each abnormal with 2.0. On the basis of the sum of these scores, we calculated the total score of CAN, which is the sum of the partial scores corresponding to each one of the four individual tests (minimum 0, maximum 8)[23].

Assessment of QT interval

Standard 12-lead ECG recordings at a paper speed of 25 mm/s were obtained. The paper recordings were then scanned to an image at high resolution (300 dpi), edited, and converted to a digital ECG recording, which was analyzed interactively using an ECG analysis program[25]. QT interval was measured from the beginning of the QRS complex to the end of the downslope of the T wave (crossing the isoelectric line). Corrected QT interval for heart rate (QTc) was calculated using Bazett's formula ($QTc = QT/\sqrt{RR}$)[26]. QT dispersion (dQT) and QTc dispersion (dQTc) were calculated as the difference between the longest and the shortest QT and QTc intervals, respectively in any of the 12 leads. All measurements were performed by a single experienced investigator who was blind to the participants' characteristics. The QTc interval was considered prolonged if it was > 440 msec (the upper normal limit commonly used).

Statistical analysis

Statistical analysis was performed using programs available in the SPSS statistical package (IBM SPSS software version 22.0 for Windows, Armonk, NY, United States) by four co-authors who have experience in statistical analysis and a biomedical statistician. All variables were tested for normal distribution of the values using the Kolmogorov-Smirnov test. Differences between groups and variables were tested by the Student's *t*-test for continuous variables, while the χ^2 test was used for categorical variables. Differences in nonparametric variables were compared using the Mann-Whitney test, while bivariate correlations were assessed by Spearman correlation for ordered variables. Multivariate linear regression analysis was performed in the patients with cirrhosis to examine for associations between QT interval parameters and the variables of interest. *P* values < 0.05 were considered statistically significant.

Table 1 Demographic and clinical characteristics as well as laboratory results of the study subjects

	Controls (n = 51)	Patients (n = 51)	P value
Male, n (%)	28 (54.9)	32 (62.7)	0.42
Age (yr)	53.8 ± 13.9	55.2 ± 14.2	0.60
BMI (kg/m^2)	26.0 ± 3.5	26.1 ± 4.4	0.95
Systolic blood pressure (mmHg)	128.3 ± 18.5	127.4 ± 27.1	0.84
Diastolic blood pressure (mmHg)	79.0 ± 10.0	76.0 ± 15.1	0.26
Heart rate (beats/min)	77.05 ± 49.82	76.74 ± 15.82	0.96
Fasting insulin (μU/mL)	10.6 (7.7-12.4)	13.3 (10.6-24.4)	< 0.001
HbA1c (%)	4.97 ± 0.50	4.03 ± 0.74	< 0.001
HOMA-IR	2.40 (1.59-3.08)	3.39 (2.74-5.50)	< 0.001
White blood cells (n/μL) × 10^3	7.4 ± 2.6	5.5 ± 4.6	0.13
Hemoglobin (g/dL)	14.8 ± 1.2	11.8 ± 2.1	< 0.001
Platelets (n/μL) × 10^3	225.766 ± 36.24	122.17 ± 94.23	< 0.001
Diabetes, n (%)	1 (2.0)	7 (13.7)	0.02
Use of diuretics, n (%)	0	14 (27.5)	< 0.001
Smoking status, n (%)			34.34
Current smokers	16 (31.4)	22 (43.1)	
Non-smokers	26 (51.0)	23 (45.1)	
Ex-smokers	9 (17.6)	5 (9.8)	

Data presented as mean ± SD or as n (%) or as median value (interquartile range). BMI: Body mass index; HbA1c: Glycated hemoglobin 1c; HOMA-IR: Homeostasis model assessment equation.

RESULTS

Demographic and clinical characteristics of the study participants

The demographic and clinical characteristics of the participants are shown in Table 1 and Table 2. Control subjects and patients with cirrhosis did not differ in terms of age, gender, BMI, arterial blood pressure, white blood cell count or smoking habits. Patients with cirrhosis had significantly higher blood glucose (P = 0.006), fasting insulin (P < 0.001) and HbA1c (P = 0.007), as well as lower Hb and platelet count levels (P < 0.001), compared to control group. A total of 27.5% of the patients received diuretics (combination of furosemide and spironolactone) (Table 1). The main causes of cirrhosis were viral hepatitis (47.1%) and alcohol abuse (33.3%), while 39% of the patients had decompensated cirrhosis (Table 2).

The association between QT-related parameters and presence as well as severity of cirrhosis

The values of all QT interval-related parameters were higher (P < 0.001) in patients with cirrhosis than those in controls (Table 3). None of the controls had a QTc interval longer than 440 msec, while 43.1% of the patients had QTc intervals longer than 440 msec. Considering 60 msec as the highest normal value for dQT, the number of individuals with dQT > 60 msec was higher in patients than in controls [n = 18 (35.3%) *vs* n = 7 (13.7%), respectively, χ^2 = 6.41, P = 0.011].

In cirrhosis group, QT parameters did not differ significantly between patients with alcoholic and non-alcoholic cirrhosis (QT: 383.8 ± 44.1 msec and 389.9 ± 36.0 msec, respectively, P = 0.98; QTc: 437.1 ± 30.5 msec and 423.7 ± 30.8 msec, P = 0.16; dQT: 65.6 ± 28.6 msec and 54.5 ± 21.2 msec, P = 0.13; dQTc: 74.4 ± 29.5 msec and 60.5 ± 23.1 msec, P = 0.12). Furthermore, patients with decompensated cirrhosis, in comparison with those with compensated cirrhosis, had longer dQTc (72.2 ± 26.6 msec *vs* 56.3 ± 22.5 msec, P = 0.03) and tended to have longer QTc (435.3 ± 30.4 msec *vs* 419.0 ± 30.1 msec, P = 0.070) as well as dQT (64.1 ± 25.4 msec *vs* 50.8 ± 20.7 msec, P = 0.053). No significant differences were found in QT (P = 0.55) between the two groups. Moreover, no significant correlations were found between the Child-Pugh score and QT (r = 0.11, P = 0.45), dQT (r = 0.20, P = 0.17), QTc (r = 0.22, P = 0.13) or dQTc (r = 0.26, P = 0.08). The same was valid for the MELD score (QT: r = -0.06, P = 0.71; dQT: r = -0.23, P = 0.12; QTc: r= 0.16, P = 0.30; dQTc: r = -0.18, P = 0.21).

Assessment of CAN

A total of 28 patients (54.9%) had CAN. All indices of cardiac ANS activity were worse and the total score, an index of the severity, of CAN was higher in patients than in controls (Table 4). Prevalence of CAN was not different between patients with compensated and decompensated cirrhosis [n = 9 (40.9%) and n = 19 (65.5%), respectively, χ^2 = 3.06, P = 0.08]. However, the severity of CAN assessed by the total score of CAN was higher in patients with decompensated than in those with compensated cirrhosis [3.0 (0.8-6.0) *vs* 4.0 (3.0-6.5), P = 0.03]. No significant correlations were found between total score of CAN and QT (r = -0.12, P = 0.40), dQT (r = 0.04, P = 0.78), QTc (r = -0.01, P = 0.98) or dQTc (r = 0.11, P = 0.43). The total score of CAN was significantly correlated with the Child-Pugh score (r = 0.33, P = 0.02) and the MELD score (r = 0.36, P = 0.01).

In addition, mean QT interval duration was not different between patients having both cirrhosis and diabetes (n = 7) and those having cirrhosis without diabetes (n = 44): 395.7 ± 41.2 msec *vs* 381.9 ±

Table 2 Clinical characteristics and associated laboratory test results of patients with cirrhosis

	n	%
Child-Pugh score	7 (5-9)	
Child-Pugh Grade A (score: 5-6)	22	43.1
B (score: 7-9)	18	35.3
C (score: 10-15)	11	21.6
MELD score	29.8 (14.4-39.6)	
Decompensated cirrhosis	29	56.9
Alcohol	17	33.3
Viral Hepatitis	24	47.1
Hepatitis B	10	*19.6*
Hepatitis C	12	*23.5*
Hepatitis B + C	2	*3.9*
Other	10	19.6
Systematic use of beta-blockers (yes)	16	31.4
Ascites (yes)	23	45.1
Esophageal varices (yes)	28	54.9
Liver biopsy	25	49.0
Histologic activity index	8.6 ± 2.9	
Disease duration (yr)	3 (0.8-7)	
INR	1.41 ± 0.41	
AST (U/L)	43.0 (34.0-66.0)	
ALT (U/L)	36.0 (24.0-50.0)	
ALP (U/L)	262.7 ± 121.8	
LDH (U/L)	392.4 ± 123.0	
γ-GT (U/L)	39.0 (28.0-76.0)	
Cholesterol (mg/dL)	175.9 ± 56.2	
Triglycerides(mg/dL)	76.0 (50.0-108.0)	
Total bilirubin (mg/dL)	1.25 (0.68-2.29)	
Direct bilirubin (mg/dL)	0.75 (0.30-1.13)	
Total proteins (g/dL)	7.5 ± 0.8	
Albumin (g/dL)	4.0 ± 0.8	
Blood potassium (meq/L)	4.2 ± 0.4	
Blood sodium (meq/L)	138.1 ± 4.6	

Data are presented as mean ± SD or as *n* (%) or as median value (interquartile range). MELD: Model for end-stage liver disease.

38.0 msec, respectively (P = 0.38). Furthermore, the values of the autonomic function tests did not differ significantly between participants having both cirrhosis and diabetes and those having cirrhosis without diabetes; deep breathing test: 1.09 ± 0.05 *vs* 1.14 ± 0.14, respectively, P = 0.29; Valsalva test: 1.40 ± 0.25 *vs* 1.32 ± 0.35, respectively, P = 0.48; lying-to-standing test: 1.09 ± 0.08 *vs* 1.08 ± 0.09, respectively, P = 0.86; orthostatic hypotension: 12.85 ± 9.50 mmHg *vs* 11.52 ± 9.73 mmHg, respectively, P = 0.73. CAN was present in 3 patients with both cirrhosis and diabetes and in 25 patients with cirrhosis but without diabetes (42.9% *vs* 56.8%, P = 0.49).

Associations between insulin resistance index (HOMA-IR) with QT-related parameters

In patients with cirrhosis, HOMA-IR values did not correlate significantly with QT (r = -0.09, P = 0.56), QTc (r = 0.18, P = 0.24), dQT (r = -0.03, P = 0.82) or dQTc (r = 0.03, P = 0.84). HOMA-IR was associated significantly with the Child-Pugh score (r = 0.43, P = 0.002) and the MELD score (r = 0.65, P < 0.001).

Table 3 Comparison of QT-related parameters between patients and controls

	Controls	Patients	*P* value
Mean QT (msec)	341.6 ± 29.4	383.9 ± 38.4	< 0.001
QT max (msec)	358.1 ± 56.6	413.5 ± 46.1	< 0.001
QT min (msec)	320.5 ± 28.1	355.4 ± 38.2	< 0.001
dQT (msec)	44.8 ± 14.2	65.6 ± 28.6	0.001
QTc (msec)	364.0 ± 20.6	428.1 ± 31.0	< 0.001
dQTc (msec)	47.6 ± 14.7	65.0 ± 25.9	< 0.001
Mean RR (msec)	863.6 ± 177.0	812.9 ± 159.8	0.13

Data are shown as mean values ± SD. QTc: Corrected QT; dQT: QT dispersion; dQTc: QTc dispersion.

Multivariate regression analysis on the association between QT interval with the study parameters

Multivariate linear regression analysis in patients with cirrhosis with QT interval as dependent variable, after controlling for age, gender and duration of cirrhosis demonstrated significant and independent associations with diagnosed diabetes [standardized regression coefficient (beta) = 0.45, P = 0.02] and use of diuretics (beta = 0.55, P = 0.03). A trend for association with HOMA-IR was observed (beta = 0.40, P = 0.058), while no significant associations were found with the Child-Pugh or the MELD score, the histology activity index, Hb, serum potassium, the total score of CAN, and previous use of beta blockers. The same analysis with either QTc, dQT or dQTc as dependent variables did not show significant associations with the aforementioned parameters.

DISCUSSION

In the present study, we found that QT and QTc intervals as well as their dispersions were substantially prolonged in patients with cirrhosis in comparison with healthy controls. In addition, we demonstrated that patients with cirrhosis were diagnosed more often with CAN.

The importance of normal liver function on preservation of the electrophysiological properties of the heart is supported by several studies that have examined the prolongation of QTc before and after liver transplantation[16]. Although the results are not unanimous, most of the data suggest that liver transplantation improved the prolonged QTc; however, the extend and the degree of the improvement was variable[16]. Thus, our data of prolonged QT interval in patients with liver cirrhosis agree and corroborate these findings.

Previous data showed abnormal QT prolongation in 37% to 84% of patients with cirrhosis of either alcoholic or nonalcoholic etiology[5,10,11,15,27,28]. However, literature data on QT dispersion in cirrhosis are scarce. Dispersion of QT interval is probably a better index of left ventricular dispersion of repolarization than QT or QTc interval and high values of dQT predict cardiovascular mortality in patients with diabetes or

Table 4 The results of the cardiac autonomic function tests in controls and patients

	Controls	Patients	*P* value
Deep breathing test (value, N/A)	1.25 ± 0.16 (48/3)	1.13 ± 0.13 (23/28)	< 0.001
Valsalva test (value, N/A)	1.45 ± 0.24 (45/6)	1.33 ± 0.25 (30/21)	0.01
Lying-to-standing test (value, N/A)	1.17 ± 0.24 (45/6)	1.08 ± 0.10 (29/22)	0.01
Systolic blood pressure fall to standing (value, N/A)	0 (0-5) (50/1)	10 (0-20) (35/16)	< 0.001
CAN, *n* (%)	3 (5.9)	28 (54.9)	< 0.001
Total score of CAN	1 (0-2)	4 (2-6)	< 0.001

Data are shown as mean ± SD or as *n* (%) or as median value (interquartile range). CAN: Cardiac autonomic neuropathy; N: Number of subjects with normal test; A: Number of subjects with abnormal test.

coronary artery disease[25]. In the literature, there are no data on the potential association between dQT and mortality in patients with liver cirrhosis. Herein we found that both dQT and dQTc were more prolonged in patients with cirrhosis. One previous study has shown that QT and dQt is prolonged in patients with cirrhosis[29], while another study shown that QT, but not dQT, is prolonged in patients with alcoholic cirrhosis in comparison with controls[30]. In contrast, in another study, no differences were found in dQT between patients with cirrhosis and controls[31]. Our findings showed that the etiology of cirrhosis was not associated with either QT or dQT prolongation.

One of the mechanisms suggested to play an important role in the pathogenesis of QT prolongation in patients with cirrhosis, is the enhanced sympathetic nervous system activity[5]. This process, which in normal subjects would reduce the QT interval, seems to participate in QT prolongation in cirrhosis. This is further elaborated with the increased circulating levels of noradrenalin, and it is an index of enhanced sympathoadrenal activity, observed in patients with advanced liver disease[10]. One would expect that the heart rate would be affected by this situation, but this is not usually the case, probably due to a downregulation of beta-adrenergic receptors[31]. Likewise, our results did not establish any substantial differences of the RR interval, which represents mean heart rate, between patients and controls. It is possible that the complex physiological changes that occur in chronic liver disease, modulate the cardiac function and may prolong the QT interval-related parameters. Moreover, although it is known that the use of propranolol reduces the risk of gastrointestinal bleeding in patients with cirrhosis[32], there are no data on the potential effect of beta-blockers on cardiovascular mortality in such patients. One systematic review and meta-analysis concluded that the use of non-selective beta-blockers was not associated with a significant increase in all-cause mortality in patients with cirrhosis and ascites or refractory ascites[33].

In our study, no differences between the cirrhotic subgroups (alcoholic *vs* non-alcoholic cirrhosis) were noticed. Thus, based upon the data originating from current study, no relationship between an increased QT interval and the cause of cirrhosis can be established. These findings are in agreement with those of previous studies and may imply that QT prolongation is a phenomenon that derives from the pathophysiology of cirrhosis itself and does not reflect abnormalities related to certain causes of cirrhosis[10]. However, in a previous study, patients with alcohol–related liver cirrhosis had a significantly (P = 0.001) higher prevalence of QTc interval prolongation than those with HBV–related liver cirrhosis[14].

Besides, no significant association was found between the values of QT parameters and the severity of cirrhosis, as assessed by the Child-Pugh or the MELD score in either univariate or multivariate analysis. In a previous study with 94 patients with cirrhosis, the Child-Pugh score and plasma norepinephrine were significant and independent determinants of QTc duration[10]. Similarly, in two other studies the prevalence of prolonged QTc increased with the severity of chronic liver disease[5,14]. The discrepancies in the results of these studies may be explained in part by differences in the studied populations. In the present study only patients with low or moderate risk of variceal bleeding were included in order to be able to discontinue safely beta-blockers, medications affecting ANS activity. However, we showed that patients with decompensated cirrhosis had longer dQTc and tended to have longer QTc as well as dQT in comparison to patients with compensated cirrhosis. This finding implies that when liver disease progresses to a point where the human body cannot overcome the cirrhosis effects, one of the clinical features of this process is the exacerbation of the cardiac electrical conductance abnormalities.

According to our findings a substantial percentage of patients have CAN, but interestingly, the severity of CAN was not associated with QT prolongation. These findings are in contrast with those seen in patients with diabetes mellitus[7]. However, our results agree with previous data in patients with cirrhosis[5,11]; thus, a previous study has shown that prolonged QTc is independent of CAN in patients with cirrhosis[5]. Moreover, diabetes was independently associated with QT in multivariate analysis confirming previous reports for association between QT prolongation in subjects with diabetes[7]. The autonomic dysfunction has been shown to correlate with the severity of liver

disease[5], a finding also observed in our study, as total score of CAN was correlated significantly with the Child-Pugh and the MELD score. Besides, patients with decompensated cirrhosis had more severe CAN than patients with compensated cirrhosis, although the prevalence of CAN was not different between the two groups. Even though experimental and clinical data suggest that ANS influence QT interval[7,34], in the present study no relationships were found between the total score of CAN and the values of QT-related parameters.

Interestingly, insulin resistance was not associated with QT-related parameters in this study, but there was a strong association between HOMA-IR and severity of cirrhosis assessed by the Child-Pugh and the MELD score. This finding implies that insulin resistance *per se* does not affect QT interval duration and that other mechanisms associated with cirrhosis affect QT interval.

Multivariate analysis demonstrated that use of diuretics was associated with QT prolongation; noteworthy, this effect was seen independently from serum potassium concentrations. This finding emphasizes the need for QT monitoring in patients with cirrhosis who are on treatment with diuretics.

It is known that diabetes is associated with higher prevalence of CAN[24] and with QT prolongation[7]. In our study, we did not find significant differences in these between patients having both diabetes and cirrhosis than those having cirrhosis without diabetes. However, the number of the participants with diabetes was small in our study and we cannot conclude robustly if presence of diabetes burdens further CAN or QT interval in patients with cirrhosis.

The strength of our study is that we examined subjects under controlled conditions and the potential confounding effects of medications, food intake and coffee consumption have been avoided. With regards, to medications, recent data suggested that propranolol administration reduces QT interval in patients with advanced liver cirrhosis waiting for liver transplantation[35]. Thus, discontinuation of beta blockers from our patients eliminated the effect of this medication on QT interval duration and allowed us to examine the net effect of the disease on QT interval duration. However, the number of the participants was not large and the study did not have enough power to support the findings. Furthermore, we did not examine for the presence of cirrhotic cardiomyopathy to look for associations between QT-related parameters and indices of systolic or diastolic function of the heart. Finally, this was a cross-sectional study and a cause and effect relationship cannot be established.

In conclusion, this study has shown that QT interval is prolonged in patients with cirrhosis compared with controls. QT prolongation is independent of the etiology and severity of cirrhosis, as well as of CAN, suggesting that this prolongation probably reflects the liver damage itself or the sympathetic nervous system predominance because of cirrhosis. Therefore, cirrhosis, even in the early stages, affects QT interval. Moreover, patients with diabetes and those on treatment with diuretics have longer QT interval independently from serum potassium levels, suggesting that they need monitoring for QT prolongation.

ARTICLE HIGHLIGHTS

Research background

Cirrhosis can affect many organs and systems of the body including cardiovascular and autonomic nervous system (ANS). Cirrhotic patients have abnormal ANS function and it is reflected in several cardiac- and vascular-related parameters such as QT interval prolongation, heart rate variability (HRV) and arterial pressure changes, all components of the so-called cardiac autonomic neuropathy (CAN). Both QT prolongation and CAN have been associated with increased cardiovascular and all-cause mortality. The findings of this study show that cirrhotic patients and, in particular those who have at the same time diabetes or who are on treatment with diuretics, have longer QT interval independently from serum electrolyte levels, suggesting that they need monitoring for QT prolongation.

Research motivation

This study has shown that patients with cirrhosis have more often CAN and QT prolongation; however, this is a cross-sectional study and a cause and effect relationship cannot be established. A prospective study is needed to examine whether patients with cirrhosis develop autonomic dysfunction and QT prolongation. Moreover, it would of interest to know the potential impact of treatment with b-blockers on QT interval or cardiac ANS activity. An important finding of this study is that the etiology of cirrhosis does not impact QT prolongation or cardiac autonomic activity.

Research objectives

The main aim of this study was to examine the impact of liver cirrhosis on QT-related parameters and on CAN. The authors' hypothesis was confirmed and implies that cardiac autonomic dysfunction and/or QT prolongation may contribute to the increased mortality in patients with cirrhosis.

Research methods

In this study, the authors managed to collect complete data related to full blood count and biochemical analyses, while the diagnosis of cirrhosis was confirmed with liver biopsies when it was indicated. The diagnosis of cardiac autonomic dysfunction was based upon robust criteria such as the battery of the tests proposed by Ewing and Clarke by determination of the HRV. QT intervals were measured using a standard 12-lead ECG recordings. Statistical analysis was performed using programs available in the SPSS statistical package by four co-authors who have experience in statistical analysis and a biomedical statistician.

Research results

In the present study, the authors found that QT and QTc intervals as well as their dispersions were substantially prolonged in patients with cirrhosis in comparison with healthy controls. In addition, the authors demonstrated that patients with cirrhosis were diagnosed more often with cardiac autonomic dysfunction. Additionally, the authors found that severity of cirrhosis does not impact QT interval but it affects severity of cardiac autonomic dysfunction.

Research conclusions

The novel finding of this study is that not only QT, but also QT dispersion is prolonged in patient with cirrhosis. Furthermore, CAN or QT prolongation is not associated with the etiology of cirrhosis. Patients with cirrhosis, especially those who have diabetes or an on treatment with diuretics should be screened for cardiac autonomic dysfunction an QT prolongation. Patients with cirrhosis have often CAN and QT prolongation. The original insights of this study are: (1) the authors measured QT dispersion, which is considered as an excellent marker of left ventricular repolarization abnormalities and better than QT prolongation, which has not been studied so far; and (2) the authors found that severity of cirrhosis affects strongly cardiac ANS activity and probably contributes to

the development of the cirrhotic myocardiopathy. The new methods used in this study is the robust methodology for the diagnosis of cardiac autonomic dysfunction and presence as well as severity of cirrhosis.

Research perspectives

The results of this study suggest that patients with cirrhosis often have QT prolongation and cardiac autonomic dysfunction and therefore, they should be screened for these comorbidities; especially those who have diabetes or an on treatment with diuretics. Future research should be directed to the potential impact of treatment with b-blockers on QT interval or cardiac ANS activity. In addition, a prospective study is needed to examine whether patients with cirrhosis develop autonomic dysfunction and QT prolongation.

REFERENCES

1 **Møller S**, Henriksen JH. Cardiovascular complications of cirrhosis. *Postgrad Med J* 2009; **85**: 44-54 [PMID: 19240290 DOI: 10.1136/gut.2006.112177]

2 **Fede G**, Privitera G, Tomaselli T, Spadaro L, Purrello F. Cardiovascular dysfunction in patients with liver cirrhosis. *Ann Gastroenterol* 2015; **28**: 31-40 [PMID: 25608575]

3 **Ruiz-del-Árbol L**, Serradilla R. Cirrhotic cardiomyopathy. *World J Gastroenterol* 2015; **21**: 11502-11521 [PMID: 26556983 DOI: 10.3748/wjg.v21.i41.11502]

4 **Ates F**, Topal E, Kosar F, Karincaoglu M, Yildirim B, Aksoy Y, Aladag M, Harputluoglu MM, Demirel U, Alan H, Hilmioglu F. The relationship of heart rate variability with severity and prognosis of cirrhosis. *Dig Dis Sci* 2006; **51**: 1614-1618 [PMID: 16927142 DOI: 10.1007/s10620-006-9073-9]

5 **Puthumana L**, Chaudhry V, Thuluvath PJ. Prolonged QTc interval and its relationship to autonomic cardiovascular reflexes in patients with cirrhosis. *J Hepatol* 2001; **35**: 733-738 [PMID: 11738100 DOI: 10.1016/S0168-8278(01)00217-3]

6 **Dümcke CW**, Møller S. Autonomic dysfunction in cirrhosis and portal hypertension. *Scand J Clin Lab Invest* 2008; **68**: 437-447 [PMID: 18609092 DOI: 10.1080/00365510701813096]

7 **Tentolouris N**, Katsilambros N, Papazachos G, Papadogiannis D, Linos A, Stamboulis E, Papageorgiou K. Corrected QT interval in relation to the severity of diabetic autonomic neuropathy. *Eur J Clin Invest* 1997; **27**: 1049-1054 [PMID: 9466135 DOI: 10.1046/j.1365-2362.1997.2300776.x]

8 **Voulgari C**, Psallas M, Kokkinos A, Argiana V, Katsilambros N, Tentolouris N. The association between cardiac autonomic neuropathy with metabolic and other factors in subjects with type 1 and type 2 diabetes. *J Diabetes Complications* 2011; **25**: 159-167 [PMID: 20708417 DOI: 10.1016/j.jdiacomp.2010.06.001]

9 **Maser RE**, Mitchell BD, Vinik AI, Freeman R. The association between cardiovascular autonomic neuropathy and mortality in individuals with diabetes: a meta-analysis. *Diabetes Care* 2003; **26**: 1895-1901 [PMID: 12766130 DOI: 10.2337/diacare.26.6.1895]

10 **Bernardi M**, Calandra S, Colantoni A, Trevisani F, Raimondo ML, Sica G, Schepis F, Mandini M, Simoni P, Contin M, Raimondo G. Q-T interval prolongation in cirrhosis: prevalence, relationship with severity, and etiology of the disease and possible pathogenetic factors. *Hepatology* 1998; **27**: 28-34 [PMID: 9425913 DOI: 10.1002/hep.510270106]

11 **Mohamed R**, Forsey PR, Davies MK, Neuberger JM. Effect of liver transplantation on QT interval prolongation and autonomic dysfunction in end-stage liver disease. *Hepatology* 1996; **23**: 1128-1134 [PMID: 8621144 DOI: 10.1002/hep.510230529]

12 **Dillon JF**, Plevris JN, Nolan J, Ewing DJ, Neilson JM, Bouchier IA, Hayes PC. Autonomic function in cirrhosis assessed by cardiovascular reflex tests and 24-hour heart rate variability. *Am J Gastroenterol* 1994; **89**: 1544-1547 [PMID: 8079935]

13 **Kim SM**, George B, Alcivar-Franco D, Campbell CL, Charnigo R, Delisle B, Hundley J, Darrat Y, Morales G, Elayi SC, Bailey AL. QT prolongation is associated with increased mortality in end stage liver disease. *World J Cardiol* 2017; **9**: 347-354 [PMID: 28515853 DOI: 10.4330/wjc.v9.i4.347]

14 **Zhao J**, Qi X, Hou F, Ning Z, Zhang X, Deng H, Peng Y, Li J, Wang X, Li H, Guo X. Prevalence, Risk Factors and In-hospital Outcomes of QTc Interval Prolongation in Liver Cirrhosis. *Am J Med Sci* 2016; **352**: 285-295 [PMID: 27650234 DOI: 10.1016/j.amjms.2016.06.012]

15 **Bal JS**, Thuluvath PJ. Prolongation of QTc interval: relationship with etiology and severity of liver disease, mortality and liver transplantation. *Liver Int* 2003; **23**: 243-248 [PMID: 12895263 DOI: 10.1034/j.1600-0676.2003.00833.x]

16 **Liu H**, Jayakumar S, Traboulsi M, Lee SS. Cirrhotic cardiomyopathy: Implications for liver transplantation. *Liver Transpl* 2017; **23**: 826-835 [PMID: 28407402 DOI: 10.1002/lt.24768]

17 **Bajaj BK**, Agarwal MP, Ram BK. Autonomic neuropathy in patients with hepatic cirrhosis. *Postgrad Med J* 2003; **79**: 408-411 [PMID: 12897221 DOI: 10.1136/pmj.79.933.408]

18 **Desmet VJ**. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis [Hepatology 1981;1:431-435]. *J Hepatol* 2003; **38**: 382-386 [PMID: 12663226 DOI: 10.1016/S0168-8278(03)00005-9]

19 **Kamath PS**, Kim WR; Advanced Liver Disease Study Group. The model for end-stage liver disease (MELD). *Hepatology* 2007; **45**: 797-805 [PMID: 17326206 DOI: 10.1002/hep.21563]

20 **American Diabetes Association**. (2) Classification and diagnosis of diabetes. *Diabetes Care* 2015; **38** Suppl: S8-S16 [PMID: 25537714 DOI: 10.2337/dc15-S005]

21 **World Medical Association**. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013; **310**: 2191-2194 [PMID: 24141714 DOI: 10.1001/jama.2013.281053]

22 **Matthews DR**, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412-419 [PMID: 3899825 DOI: 10.1007/BF00280883]

23 **Ewing DJ**, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care* 1985; **8**: 491-498 [PMID: 4053936 DOI: 10.2337/diacare.8.5.491]

24 **Pop-Busui R**, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, Sosenko JM, Ziegler D. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. *Diabetes Care* 2017; **40**: 136-154 [PMID: 27999003 DOI: 10.2337/dc16-2042]

25 **Psallas M**, Tentolouris N, Papadogiannis D, Doulgerakis D, Kokkinos A, Cokkinos DV, Katsilambros N. QT dispersion: comparison between participants with Type 1 and 2 diabetes and association with microalbuminuria in diabetes. *J Diabetes Complications* 2006; **20**: 88-97 [PMID: 16504837 DOI: 10.1016/j.jdiacomp.2005.05.012]

26 **Luo S**, Michler K, Johnston P, Macfarlane PW. A comparison of commonly used QT correction formulae: the effect of heart rate on the QTc of normal ECGs. *J Electrocardiol* 2004; **37** Suppl: 81-90 [PMID: 15534815 DOI: 10.1016/j.jelectrocard.2004.08.030]

27 **Trevisani F**, Merli M, Savelli F, Valeriano V, Zambruni A, Riggio O, Caraceni P, Domenicali M, Bernardi M. QT interval in patients with non-cirrhotic portal hypertension and in cirrhotic patients treated with transjugular intrahepatic porto-systemic shunt. *J Hepatol* 2003; **38**: 461-467 [PMID: 12663238 DOI: 10.1016/S0168-8278(03)00057-6]

28 **Cichoż-Lach H**, Tomaszewski M, Kowalik A, Lis E, Tomaszewski A, Lach T, Boczkowska S, Celiński K. QT Interval Prolongation and QRS Voltage Reduction in Patients with Liver Cirrhosis. *Adv Clin Exp Med* 2015; **24**: 615-622 [PMID: 26469105 DOI: 10.17219/acem/28681]

29 **Tuttolomondo A**, Buttà C, Casuccio A, Di Raimondo D, Serio A, D'Aguanno G, Pecoraro R, Renda C, Giarrusso L, Miceli G, Cirrincione A, Pinto A. QT Indexes in Cirrhotic Patients: Relationship with Clinical Variables and Potential Diagnostic Predictive Value. *Arch Med Res* 2015; **46**: 207-213 [PMID:

25843561 DOI: 10.1016/j.arcmed.2015.03.008]
30 **Day CP**, James OF, Butler TJ, Campbell RW. QT prolongation and sudden cardiac death in patients with alcoholic liver disease. *Lancet* 1993; **341**: 1423-1428 [PMID: 8099138 DOI: 10.1016/0140-6736(93)90879-L]
31 **Hansen S**, Møller S, Bendtsen F, Jensen G, Henriksen JH. Diurnal variation and dispersion in QT interval in cirrhosis: relation to haemodynamic changes. *J Hepatol* 2007; **47**: 373-380 [PMID: 17459513 DOI: 10.1016/j.jhep.2007.03.013]
32 **Pagliaro L**. Lebrec D, Poynard T, Hillon P, Benhamou J-P. Propranolol for prevention of recurrent gastrointestinal bleeding in patients with cirrhosis. A controlled study [N Engl J Med 1981;305:1371-1374]. *J Hepatol* 2002; **36**: 148-150 [PMID: 11830324 DOI: 10.1056/NEJM198112033052302]
33 **Chirapongsathorn S**, Valentin N, Alahdab F, Krittanawong C, Erwin PJ, Murad MH, Kamath PS. Nonselective β-Blockers and Survival in Patients With Cirrhosis and Ascites: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2016; **14**: 1096-1104.e9 [PMID: 26829026 DOI: 10.1016/j.cgh.2016.01.012]
34 **Aytemir K**, Aksöyek S, Ozer N, Gürlek A, Oto A. QT dispersion and autonomic nervous system function in patients with type 1 diabetes. *Int J Cardiol* 1998; **65**: 45-50 [PMID: 9699930 DOI: 10.1016/S0167-5273(98)00091-6]
35 **Kim YK**, Hwang GS, Shin WJ, Bang JY, Cho SK, Han SM. Effect of propranolol on the relationship between QT interval and vagal modulation of heart rate variability in cirrhotic patients awaiting liver transplantation. *Transplant Proc* 2011; **43**: 1654-1659 [PMID: 21693252 DOI: 10.1016/j.transproceed.2011.02.017]

P- Reviewer: Abadi ATB, Liu DY, McMillin MA, Morini S
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ISSN 2150-5330 (online)

World Journal of *Gastrointestinal Pathophysiology*

World J Gastrointest Pathophysiol 2018 September 29; 9(2): 37-58

Published by Baishideng Publishing Group Inc

WJGP

World Journal of Gastrointestinal Pathophysiology

Contents

Volume 9 Number 2 September 29, 2018

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World Journal of Gastrointestinal Pathophysiology is now abstracted and indexed in PubMed, PubMed Central, China National Knowledge Infrastructure (CNKI), and Superstar Journals Database.

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NAME OF JOURNAL
World Journal of Gastrointestinal Pathophysiology

ISSN
ISSN 2150-5330 (online)

LAUNCH DATE
April 15, 2010

EDITORIAL OFFICE
Jin-Lei Wang, Director
World Journal of Gastrointestinal Pathophysiology
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA

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PUBLICATION DATE
September 29, 2018

INSTRUCTIONS TO AUTHORS
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World Journal of Gastrointestinal Pathophysiology

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World J Gastrointest Pathophysiol 2018 September 29; 9(2): 37-46

DOI: 10.4291/wjgp.v9.i2.37

ISSN 2150-5330 (online)

REVIEW

Acinar cell injury induced by inadequate unfolded protein response in acute pancreatitis

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Author contributions: Barrera K, Stanek A, Tenner S and Huan C conceptualized, designed and drafted the study; Barrera K, Stanek A, Okochi K, Niewiadomska Z, Tenner S and Huan C reviewed literatures; Barrera K, Stanek A, Mueller C, Ou P, John D, Alfonso AE, Tenner S and Huan C revised and edited the manuscript.

Conflict-of-interest statement: No potential conflicts of interest.

Manuscript source: Invited Manuscript

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Received: June 21, 2018
Peer-review started: June 21, 2018
First decision: July 23, 2018
Revised: July 24, 2018
Accepted: August 21, 2018
Article in press: August 21, 2018
Published online: September 29, 2018

Abstract

Acute pancreatitis (AP) is an inflammatory disorder of pancreatic tissue initiated in injured acinar cells. Severe AP remains a significant challenge due to the lack of effective treatment. The widely-accepted autodigestion theory of AP is now facing challenges, since inhibiting protease activation has negligible effectiveness for AP treatment despite numerous efforts. Furthermore, accumulating evidence supports a new concept that malfunction of a self-protective mechanism, the unfolded protein response (UPR), is the driving force behind the pathogenesis of AP. The UPR is induced by endoplasmic reticulum (ER) stress, a disturbance frequently found

in acinar cells, to prevent the aggravation of ER stress that can otherwise lead to cell injury. In addition, the UPR's signaling pathways control NFκB activation and autophagy flux, and these dysregulations cause acinar cell inflammatory injury in AP, but with poorly understood mechanisms. We therefore summarize the protective role of the UPR in AP, propose mechanistic models of how inadequate UPR could promote NFκB's pro-inflammatory activity and impair autophagy's protective function in acinar cells, and discuss its relevance to current AP treatment. We hope that insight provided in this review will help facilitate the research and management of AP.

Key words: Acute pancreatitis; Endoplasmic reticulum stress; Unfolded protein response; Acinar cell injury; Autophagy

Core tip: The widely-accepted autodigestion theory of acute pancreatitis (AP) has been considerably modified by the recent recognition of endoplasmic reticulum (ER) stress-induced unfolded protein response (UPR) as an essential self-protective activity in acinar cells. Inadequate UPR, however, leads to acinar cell injury in AP with elusive mechanisms. We review the relevant literature and propose mechanistic models with the hope of facilitating the research required for the development of effective AP treatment.

Barrera K, Stanek A, Okochi K, Niewiadomska Z, Mueller C, Ou P, John D, Alfonso AE, Tenner S, Huan C. Acinar cell injury induced by inadequate unfolded protein response in acute pancreatitis. *World J Gastrointest Pathophysiol* 2018; 9(2): 37-46 Available from: URL: http://www.wjgnet.com/2150-5330/full/v9/i2/37.htm DOI: http://dx.doi.org/10.4291/wjgp.v9.i2.37

INTRODUCTION

Acute pancreatitis (AP) is one of the most common gastrointestinal disorders leading to hospitalization in the United States, accounting for more than 270000 hospital admissions and costing 2.6 billion dollars per year[1]. More than 75% of AP cases are associated with alcohol consumption and gallstones, and up to 20% of AP patients have a severe form with a mortality rate between 10% to 30%[2]. Severe complications of AP include progression to pancreatic necrosis, bacteremia, sepsis, splenic vein thrombosis, and respiratory failure. Current management strategies for AP treatment, such as aggressive hydration, endoscopic intervention for biliary obstructive disease and pancreatic necrosectomy, have limited beneficial effects on disease progression and results[3]. Therapy that can effectively block the progression of acinar injury before it results in severe complications is still missing. This is largely due to the poor understanding of the molecular dysregulation that leads to irreversible inflammatory injury in acinar cells, despite a wide range of efforts that have been made to define the mechanisms of AP.

In 1896, Hans Chiari, based on his postmortem observations, originally proposed that pancreatitis is a process of autodigestion of the pancreas when "the organ succumbs to its own digestive properties"[4]. Nearly a century later, this concept gained acceptance when elevated levels of trypsin and other proteases were observed in AP animal models, and when mutations in the trypsinogen gene were found in patients with hereditary pancreatitis[5,6]. Further observation of the co-localization of lysozyme with secretory granules in acinar cells also supported the belief that intracellular activation of trypsinogen by lysozyme was the mechanism of autodigestion[7].

Based on the autodigestion theory, several protease inhibitors were developed for AP treatment over the past 50 years[5]. Although few human and animal studies showed beneficial activities of this strategy, including the prophylactic effects of Gabexate on post-endoscopic retrograde cholangiopancreatography AP[8], larger clinical trials failed to demonstrate the effectiveness of these inhibitors in patients with AP. This was thought to be due to the late timing in which these protease inhibitors were provided to patients, which was typically hours after the onset of AP[5].

Animal studies of trypsinogen-deficient mice, however, generated more evidence challenging the trypsinogen-induced autodigestion theory. Despite being deficient in major trypsinogen activity, these mice were still able to develop AP and chronic pancreatitis (CP)[9,10]. Furthermore, in some hereditary pancreatitis patients, trypsinogens encoded by mutated trypsinogen genes had unaltered trypsin activity, but signs of defective protein folding in the endoplasmic reticulum (ER) lumen[11]. Thus, acinar cell injury in AP is not necessarily the result of premature intracellular activation of trypsinogen as previously thought. Other dysregulated cellular activities are likely responsible for triggering injury in acinar cells.

ER STRESS IS A COMMON DISTURBANCE THAT INITIATES ACINAR CELL INJURY

The ER is a multifunctional organelle that stores calcium and metabolizes lipids and carbohydrates, but is principally responsible for protein folding and processing in cells. ER stress is a malfunctioning condition characterized by the accumulation of unfolded and misfolded proteins in the ER lumen[12]. Acinar cells, as the primary producers of digestive enzymes, have abundant ER that enables the highest rate of protein synthesis and processing among the mature cells in the body. This unique feature, however, makes acinar cells particularly susceptible to AP risk factor-induced ER stress[12]. Severe and enduring ER stress can cause irreversible cellular damage associated with an increase of intracellular reactive oxygen species, release of cytochrome c from mitochondria, induction of

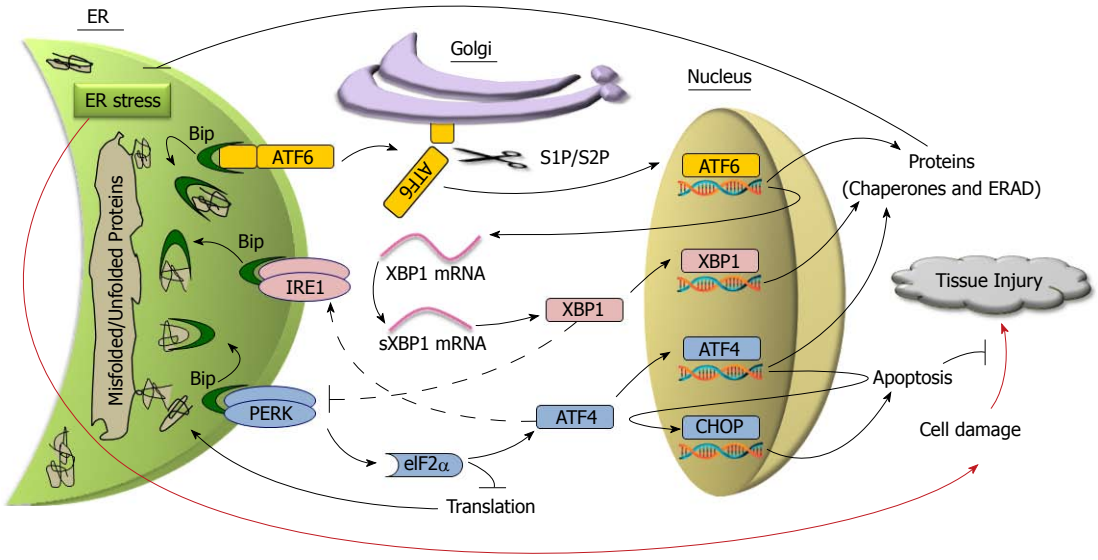

Figure 1 Unfolded protein response protects against tissue injury by relieving endoplasmic reticulum stress. Red arrows represent the pathways that lead to tissue injury. Dotted lines represent the interactions with unclarified mechanisms.

caspase 12-mediated apoptosis, blockade of autophagic flux, promotion of NFκB-mediated inflammation, and perturbation of calcium-regulated signaling[12-15]. Damaged acinar cells then inevitably promote a local inflammatory response that can attenuate self-protective activities in the remaining intact acinar cells and thereby extend the local injury, which may eventually escalate mild AP to severe AP.

UPR PROTECTS AGAINST ACINAR CELL INJURY BY RELIEVING ER STRESS

First described by Sambrook's group in 1988, the unfolded protein response (UPR) is a concerted effort made by the cell to intricately alleviate ER stress that would otherwise significantly threaten normal cellular functions[16]. Failure to counterbalance ER stress by the UPR has been implicated in a broad range of diseases including diabetes, neurodegeneration, cancer, pulmonary fibrosis, cardiac disease and inflammatory disorders such as AP, identifying the UPR as an essential self-protective mechanism[17]. In acinar cells, due to the high susceptibility to ER stress, the UPR is therefore decisive in maintaining cellular homeostasis[12-15].

As illustrated in Figure 1, activation of the UPR is initiated by three ER transmembrane proteins, including protein kinase RNA-like ER kinase (PERK), inositol requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6), and each of them activates a different UPR signaling pathway[18]. In the absence of ER stress, these three proteins are bound to a chaperone protein called binding immunoglobulin protein (BiP) that holds them in inactive states on the ER membrane. In stressed ER when unfolded proteins accumulate, however, BiP releases PERK, IRE1 and ATF6 in order to bind to unfolded proteins to help with their folding. The dissociation of BiP triggers activation of PERK and IRE1 *via* their auto-phosphorylation, and enables ATF6 to translocate to the Golgi apparatus where it is cleavage-activated by proteases. Activated PERK, IRE1 and ATF6 then, *via* different sequential proceedings, turn on diverse UPR activities, which include: Reducing total protein production by inhibiting translation, eliminating misfolded and unfolded proteins in the ER lumen through ER-associated degradation (ERAD), and increasing the folding capability in the ER by producing more chaperone proteins. Of note, the three UPR regulatory pathways appear to be distinct yet interactive in maintaining homeostasis. Dysregulation of the UPR pathways by AP risk factors, however, is considered as the cause that leads to acinar cell injury[19,20].

PERK/eIF2/ATF4

PERK signaling controls general protein translation and cell apoptosis in response to ER stress. Activated PERK phosphorylates eukaryotic initiation factor-2α (eIF2α), which inhibits general protein translation by interfering with the formation of the initiation complex at ribosomes. This prevents further accumulation of unfolded and misfolded proteins in the ER lumen[21]. Although it represses global protein translation, phosphorylated eIF2α preferentially promotes the translation of ATF4, which activates the transcription of other UPR genes, including proteins needed for carrying out protein folding and ERAD[22], and enhances the IRE1 pathway[23]. In addition, upregulated ATF4 signaling can activate apoptosis *via* transcriptional regulation of C/EBP homologous protein (CHOP), a transcription factor that directs ER stress-induced apoptosis[24].

Alternated PERK pathway is associated with various disorders including diabetes, metabolic and inflammatory diseases, and cancers[17,25]. Loss-of-function mutations in PERK cause Wolcott-Rallison syndrome manifesting as early onset type 1 diabetes, epiphyseal dysplasia,

osteopenia, mental retardation, and hepatic and renal dysfunction[26]. The involvement of multiple organs in Wolcott-Rallison syndrome indicates the broad range of PERK's protective activities in the body. On the other hand, over-activated PERK can be harmful. In prion-infected mice, excessive and long-term ER stress-induced over-activation of the PERK/eIF2α/ATF4 pathway led to neurodegeneration[27]. Based on these findings, efforts have been made to target PERK as a potential therapeutic strategy in ER stress-related diseases[28].

Interestingly, although PERK, ATF4 and CHOP are sequentially activated in the same pathway, each deficiency causes different phenotypes in the mouse pancreas, indicating that their functions are not fully overlapped in acinar cells. Similar to Wolcott-Rallison syndrome in humans, PERK-deficient mice present with significant pancreatic atrophy associated with increased pancreatic cell death early after their birth[29]. While PERK is required for both secretory homeostasis and survival in β cells, in acinar cells it is only needed for maintaining the viability, but not for enzyme synthesis and secretion[29,30]. In line with this, no ER stress is observed in PERK-deficient acinar cells[29]. ATF4-deficient mice, however, have severely underdeveloped exocrine pancreata with a reduced numbers of acinar cells, indicating a development role of ATF4 in acinar cells[29]. In contrast to PERK-deficient mice and ATF4-deficient mice, CHOP-deficient mice have a completely normal pancreas[31]. Activation of PERK/eIF2α/ATF4 is upregulated in injured acinar cells, leading to the inhibition of general translation and the expression of pro-apoptotic CHOP[32,33]. Increased CHOP is found to be protective in a severe AP animal model, likely because it can direct the fate of injured acinar cells toward less harmful apoptosis instead of more destructive necrosis[31].

IRE1/XBP1

On the membrane of stressed ER, the ribonuclease function of IRE1 is activated to excise an intron from the mRNA of X-box binding protein 1 (XBP1), whose expression is regulated by ATF6[34,35]. Spliced XBP1 mRNA (sXBP1) encodes an active form of XBP1 that activates the transcription of chaperones and ERAD components (Figure 1). Interestingly, in addition to its ribonuclease function, IRE1 has a kinase domain that regulates non-UPR signaling in response to ER stress, such as the activation of nuclear factor kappa light chain enhancer of activated B cells (NFκB)[36].

The IRE1/XBP1 pathway is dysregulated in multiple ER stress-associated human diseases[25], which led to mechanistic studies of the IRE1/XBP1 pathway in different animal models. In neurodegenerative disease models, XBP1 appeared to be pathogenic in amyotrophic lateral sclerosis and Huntington's disease *via* the inhibition of autophagy[37,38]. In Alzheimer's disease and Parkinson's disease models, however, XBP1-mediated UPR was neuroprotective[39,40]. Interestingly, unlike PERK, XBP1 was dispensable in prion-related disorders[27,41]. In gastrointestinal disorders, IRE1 alleviated ER perturbations in intestinal epithelial cells in inflammatory bowel disease[42], and XBP1 enhanced fibrogenic activity in hepatic stellate cells in a steatosis model[43]. XBP1 was also important for glucose and lipid homeostasis, and linked obesity to type 2 diabetes[44,45]. Thus, the IRE1/XBP1 pathway has distinctive roles in disease progression depending on the pathogenesis. Accordingly, IRE1/XBP1 inhibitors and activators have been developed for disease treatment[28,46]. Still, more extensive and rigorous pre-clinical studies are needed to predict their effectiveness in the clinical setting.

The IRE1/XBP1 pathway is vital for pancreas development, as deficiency of IRE1 or XBP1 impaired exocrine pancreas development in Xenopus and mice[47-49]. In normal pancreatic acinar cells, the IRE1/XBP1 pathway has a basal activity level[50]. Inhibition of IRE1 or XBP1 reduced spontaneous digestive enzyme secretion in acinar cells[13,51], indicating that unlike PERK, the IRE1/XBP1 pathway is required for ordinary digestive function. Notably, inhibition of IRE1/XBP1 led to the over-activation of PERK in acinar cells, and over-activated PERK was associated with diminished XBP1 in AP[32,33]. Although XBP1 expression is transcriptionally regulated by ATF6[35], how XBP1 expression diminishes in AP remains unknown. Intriguingly, unlike in AP, XBP1 is elevated in CP along with other UPR elements[52]. These results suggest that diminished XBP1 could be an early event in the chain of UPR pathway dysregulation in AP.

ATF6

ER stress induces Golgi translocation and cleavage-activation of ATF6. The two proteases that sequentially cleave ATF6 on the Golgi are site one and two proteases (S1P and S2P), which also regulate cholesterol and fatty acid synthesis in the liver *via* cleavage-activation of sterol regulatory element-binding proteins (SREBPs)[53,54]. Cleaved ATF6 then enters the nucleus and activates the transcription of other genes required for UPR activities[18]. Compared to PERK and IRE1 that regulate diverse cellular activities, ATF6 mainly activates the transcription of chaperones and ERAD components. Notably, ATF6 also activates the transcription of XBP1[35], whose activity could in turn inhibit PERK activation[32,33]. Thus, ATF6 appears to initiate interactions among the three UPR pathways.

Studies have shown that ATF6-regulated UPR modulates hepatic and neurologic processes. In liver, ATF6 controls gluconeogenesis and blocks ER stress-induced steatosis[55,56]. In the nervous system, ATF6 is neuroprotective in Huntington's disease *via* the activation of UPR's pro-survival activities[57]. Mutations in ATF6 increase the susceptibility to ER stress-induced damage, which underlies the pathogenesis of the visual disorder achromatopsia[58]. Despite the recognized roles of ATF6 in diseases, no drugs have been developed to specifically target ATF6, and only a couple of S1P inhibitors have been used to experimentally reduce lipid synthesis and viral propagation[59,60].

Among PERK, IRE1 and ATF6, ATF6 seems to have the highest sensitivity to ER stress in acinar cells. This is

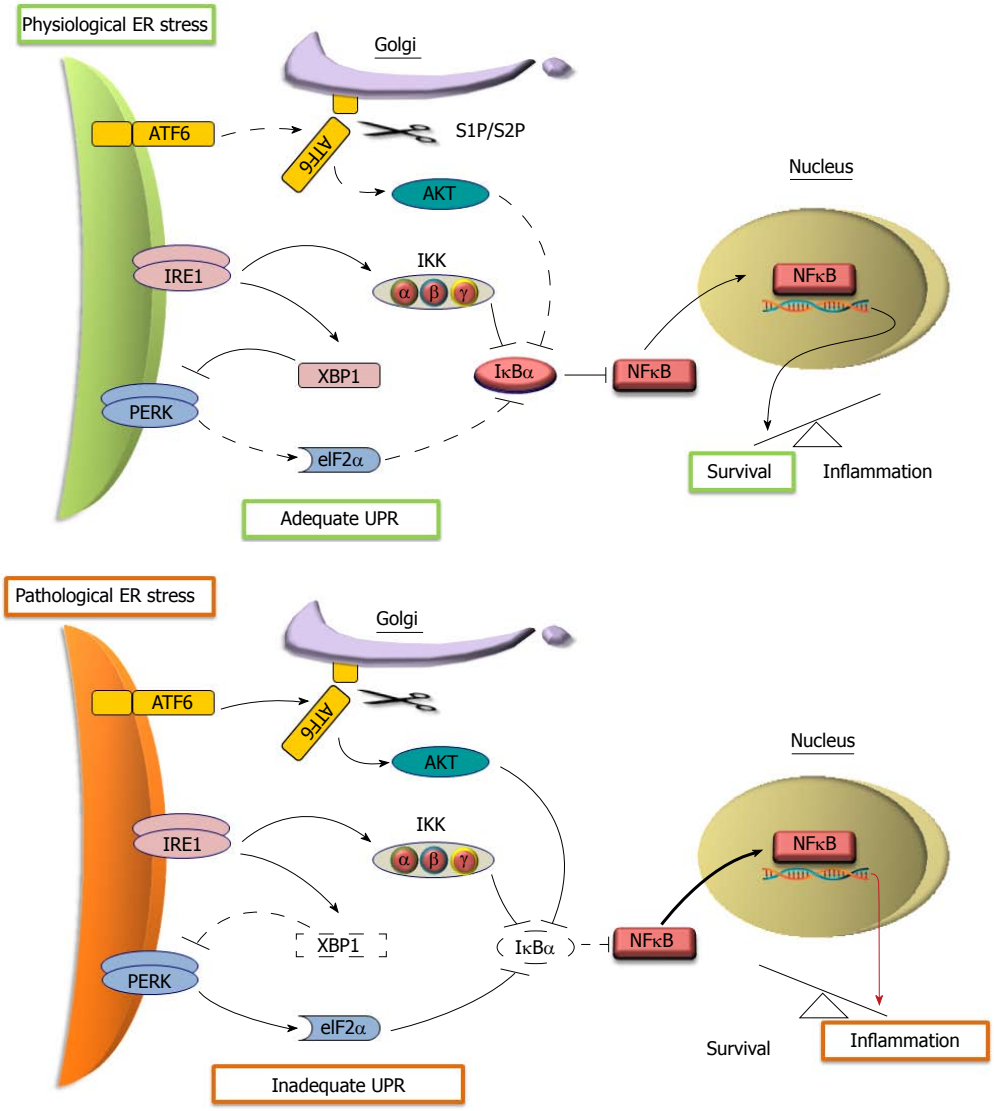

Figure 2 Proposed models of NFκB activation by the unfolded protein response in response to endoplasmic reticulum stress. In physiological endoplasmic reticulum (ER) stress, adequate unfolded protein response (UPR) activates basal levels of NFκB nuclear translocation that trigger the transcription of pro-survival genes (upper panel). In pathological ER stress, NFκB upregulated by inadequate UPR activates the transcription of pro-inflammatory genes (lower panel). Arrows and lines represent active (solid) and ineffective (dotted) signaling. Thick and red arrows symbolize enhanced interactions and pro-inflammatory signaling, respectively.

because ATF6 nuclear translocation was observed much earlier than upregulation of BiP, XBP1 mRNA splicing or CHOP expression in a rat AP model[50]. Highly increased ATF6, along with phosphorylation of PERK and eIF2 and upregulation of CHOP, was also observed in a mouse binge-drinking model[61]. We consistently found increased cleavage of ATF6 in acinar cells in response to cerulein-induced ER stress, and confirmed that S1P-mediated cleavage-activation of ATF6 was required for the protection of acinar cells in AP[62]. Thus, the ATF6 pathway is a potential target for AP treatment.

UPR IN THE REGULATION OF NFκB-MEDIATED INFLAMMATORY RESPONSE IN AP

Although knowledge about inflammatory regulation is growing rapidly, how cells initiate inflammation in response to intracellular disturbances is still poorly understood. Interestingly, signaling pathways that control NFκB-mediated inflammatory responses and the UPR were found to be integrated, strongly suggesting that they originate through a common mechanism[63]. As shown in Figure 2, activated eIF2α in the PERK pathway inhibits the translation of both NFκB and its inhibitor IκB, which results in the activation of NFκB since IκB has a much shorter half-life compared to NFκB[63]. Additionally, the kinase function of IRE1 can phosphorylate IκB kinase (IKK) in response to stress, resulting in the degradation of IκBα and subsequent NFκB activation[36]. ATF6 may also activate NFκB *via* AKT-mediated degradation of IκB[64].

In the pancreas, evidence has been mounting in support of a dual role of NFκB in the regulation of survival and inflammation in acinar cells. Basal NFκB activity is considered as prosurvival in acinar cells, while highly active

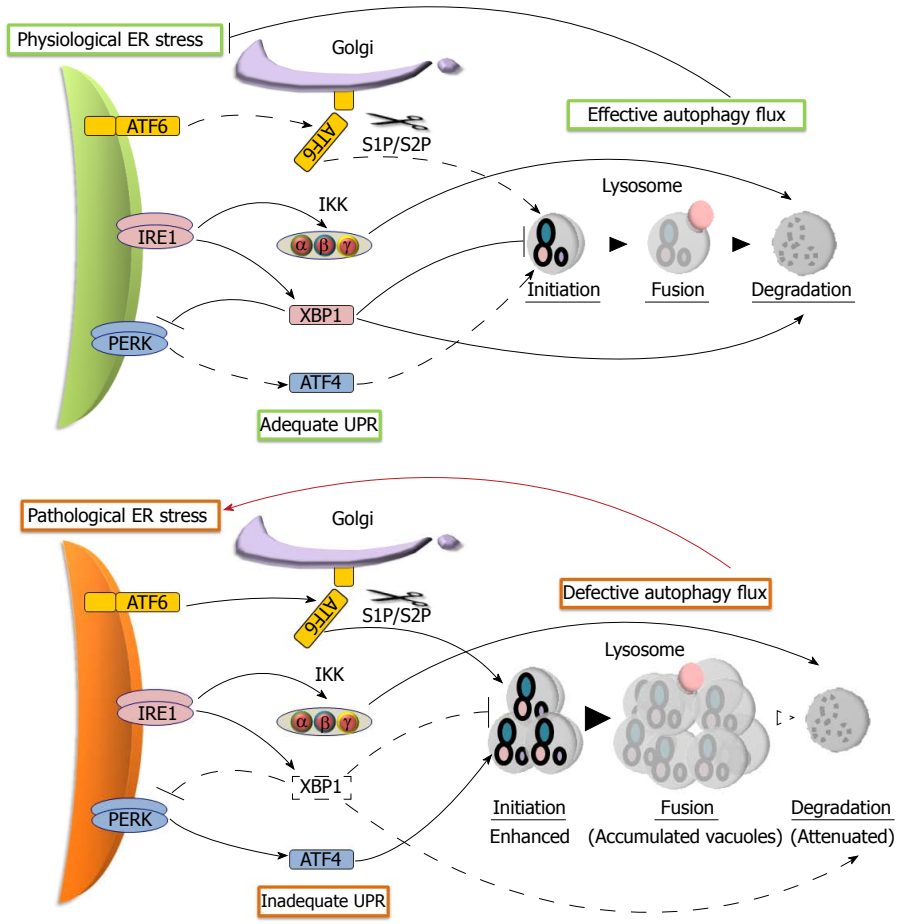

Figure 3 Proposed models of autophagy flux regulation by the unfolded protein response in response to endoplasmic reticulum stress. In physiological endoplasmic reticulum (ER) stress, adequate unfolded protein response (UPR) maintains effective autophagy flux that relieves ER stress (upper panel). In pathological ER stress, however, inadequate UPR causes the accumulation of intracellular vacuoles, which further aggravates ER stress (lower panel). Arrows and lines represent active (solid) and ineffective (dotted) signaling. Thick and red arrows symbolize enhanced interactions and harmful activity, respectively.

NFκB favors the proinflammatory "arm"[65]. The prosurvival activity of NFκB in acinar cells is evidenced by worsened AP in the mouse pancreas with a loss-of-function mutation in the NFκB subunit p65[66], as well as the ameliorated AP in IκBα-mutated mice that have increased basal NFκB activity[67]. However, the proinflammatory effect becomes dominant when NFκB is over-activated in AP[68,69]. As shown in Figure 2, we propose that adequate UPR induces basal NFκB activity to enhance the survival of acinar cells, since besides IRE1/IKK, neither PERK/eIF2α nor ATF6/AKT are effective in inducing the degradation of IκBa. In dysregulated UPR, however, all three pathways are activated to effectively promote IκBα degradation. This results in significantly upregulated NFκB activity, which promotes the inflammatory response in AP. In support of this model, a study has shown that maximized NFκB activation can be induced by the cooperation between PERK/eIF2α-mediated translation repression and IRE1-mediated phosphorylation of IKK in response to ER stress[70]. In addition, the inhibition of AKT attenuated pancreas inflammation in a severe AP model associated with reduced activation of NFκB[71], supporting the possible role of ATF6-regulated AKT in the over-activation of NFκB in acinar cells.

UPR IN THE REGULATION OF AUTOPHAGY IN AP

Autophagy is another fundamental protective activity, whose impairment has been considered as a point of convergence in the multiple deranged pathways of AP. Autophagy helps relieve ER stress by regulating cellular degradation[72]. Impaired autophagy in AP is characterized by defective autophagic flux, with the accumulation of large autophagic vacuoles manifesting as vacuolization in acinar cells. As shown in Figure 3, the pathways in adequate UPR help maintain autophagic flux. XBP1 prevents the accumulation of autophagic vacuoles by repressing the induction of autophagy and facilitating the processing of cathepsin, a lysosomal protease required for the activation of acid hydrolases in autophagic vacuoles[37,38,73,74]. The promotion of autophagic protein degradation by IRE1-activated IKK also facilitates autophagic flux in acinar cells, since both IRE1-deficient mice and IKK-deficient mice have spontaneous acinar cell vacuolization[49,75]. In addition to IRE1/XBP1, the role of

ATF4 and ATF6 in the promotion of autophagy cannot be excluded, since they activate the transcription of autophagy genes[76,77]. In AP, however, we propose that the initiation of autophagy is significantly enhanced by a combined effect of diminished XBP1 with upregulated ATF6 and ATF4, while the protein degradation is attenuated because of the lack of enough XBP1 to effectively process cathepsins. These dysregulations of autophagic flux could synergistically induce the vacuolization of acinar cells, which further aggravates pathogenic ER stress in AP (Figure 3).

Thus, multiple lines of evidence support a model of AP in which the protective UPR is undesirably transformed into a driving force behind pathogenic ER stress, proinflammatory NFκB activation and defective autophagy in injured acinar cells. Further validation of this model could help elucidate the pathogenesis of AP.

UPR AND AP MANAGEMENT

Recognition of the failure of inadequate UPR to relieve ER stress as an initiation fact of acinar cell injury in AP can make clinicians more cautious of using the medications that impair the UPR in patients with AP risk. For example, Bortezomib, an ERAD inhibitor used in the treatment of patients with multiple myeloma, induced AP[78].

Understanding the UPR in AP helps address the concern of the replacement of total parenteral nutrition with enteral nutrition in current AP management. Total parenteral nutrition was a universal management therapy for both mild and severe AP in the 1980s and 1990s. This was because total parenteral nutrition was thought to alleviate the burden on injured acinar cells in AP, since acinar secretion in healthy individuals induced by enteral nutrition can be avoided with parenteral nutrition[79]. In AP patients, however, enteral nutrition may not necessarily increase the enzyme production in acinar cells, since the PERK pathway that blocks the synthesis of digestive enzymes is highly activated in acinar cells. Indeed, multiple studies have proven that enteral nutrition does not worsen the pancreatic injury in AP patients, but has significantly decreased the risk of intestinal infection associated with total parenteral nutrition[3].

In addition, some strategies in current AP management alleviate the inflammatory microenvironment that otherwise could worsen ER stress and dysregulated UPR in AP. For example, early aggressive hydration is somehow effective in preventing serious complications, such as pancreatic necrosis[3]. The considered underlying mechanisms include resolving the hypoxia, nutrient deprivation, and pH changes in the inflamed AP tissues that may aggravate the dysregulation of the UPR in injured acinar cells. Moreover, the shifting concept of surgical management of pancreatic necrosis also supports the importance of the microenvironment in acinar injury. Open necrosectomy was previously practiced widely for necrotizing pancreatitis. However, studies have shown that the mortality in stable patients with infected necrosis can be significantly reduced if necrosectomy is delayed until the necrosis is walled-off by fibrous tissue. This favorable outcome is likely associated with the recovery of the UPR in residual acinar cells. Similarly, the minimally-invasive step-up approach that can efficiently minimize the surgical trauma and stress in residual acinar cells has been shown to be superior to open necrosectomy for necrotizing pancreatitis[80].

The finding of dysregulated UPR in AP also provides potential targets for new pharmacological intervention. During the pathogenesis of AP, the initial chain of events in the acinar cells that lead to the clinical presentation of AP are quite distant to the patient presenting in the emergency room. Hours later, when the patient presents, it may seem to be too late for pancreatic function-targeted interventions to be beneficial[5]. However, this is not likely to be true for several reasons. The majority of patients have mild disease upon admission and only progress to severe disease over the next 24-48 h[3]. Additionally, few patients who develop necrosis of the pancreas have this finding on admission computed tomography. Most complications of the disease, such as pulmonary edema, sepsis and renal failure, develop later in the course of the disease. Considering the pattern of clinical progression and the ongoing acinar cell destruction seen as pancreatic necrosis evolves, the events in the acinar cell that cause AP represent an important target for pharmacological intervention. This is supported by the fact that up to 80% of AP cases are self-limited by self-protective mechanisms[2,3], such as the UPR that alleviates the disturbances in acinar cells. Therefore, targeting the UPR seems to be a reasonable strategy to prevent the aggravation of pancreatic injury and inflammation in AP when the patient presents.

CONCLUSION

In summary, we have proposed that dysregulated UPR plays a decisive role in the pathogenesis of AP. Of note, in comparison to the rapidly-growing research on other ER stress-associated disorders such as neurodegenerative diseases, studies of how AP risk factors impair the UPR and lead to acinar cell injury are very limited. In order to improve AP management, more efforts and resources are needed to identify the UPR pathway as a potential target for therapeutic intervention in AP.

REFERENCES

1 **Peery AF**, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, Gangarosa LM, Thiny MT, Stizenberg K, Morgan DR, Ringel Y, Kim HP, DiBonaventura MD, Carroll CF, Allen JK, Cook SF, Sandler RS, Kappelman MD, Shaheen NJ. Burden of gastrointestinal disease in the United States: 2012 update. *Gastroenterology* 2012; **143**: 1179-1187.e3 [PMID: 22885331 DOI: 10.1053/j.gastro.2012.08.002]

2 **Steinberg W**, Tenner S. Acute pancreatitis. *N Engl J Med* 1994; **330**: 1198-1210 [PMID: 7811319 DOI: 10.1056/NEJM199404283301706]

3 **Tenner S**, Baillie J, DeWitt J, Vege SS; American College of Gastroenterology. American College of Gastroenterology guideline: management of acute pancreatitis. *Am J Gastroenterol* 2013; **108**: 1400-15; 1416 [PMID: 23896955]

4 **Chiari H.** Über die Selbstverdauung des menschlichen Pankreas. *Zeitschrift für Heilkunde* 1896; **17**: 69-96

5 **Singh VP**, Chari ST. Protease inhibitors in acute pancreatitis: lessons from the bench and failed clinical trials. *Gastroenterology* 2005; **128**: 2172-2174 [PMID: 15940654 DOI: 10.1053/j.gastro.2005.03.087]

6 **Whitcomb DC**, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK Jr, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996; **14**: 141-145 [PMID: 8841182 DOI: 10.1038/ng1096-141]

7 **Willemer S**, Bialek R, Adler G. Localization of lysosomal and digestive enzymes in cytoplasmic vacuoles in caerulein-pancreatitis. *Histochemistry* 1990; **94**: 161-170 [PMID: 2358374 DOI: 10.1007/BF02440183]

8 **Cavallini G**, Tittobello A, Frulloni L, Masci E, Mariana A, Di Francesco V. Gabexate for the prevention of pancreatic damage related to endoscopic retrograde cholangiopancreatography. Gabexate in digestive endoscopy--Italian Group. *N Engl J Med* 1996; **335**: 919-923 [PMID: 8786777 DOI: 10.1056/NEJM199609263351302]

9 **Dawra R**, Sah RP, Dudeja V, Rishi L, Talukdar R, Garg P, Saluja AK. Intra-acinar trypsinogen activation mediates early stages of pancreatic injury but not inflammation in mice with acute pancreatitis. *Gastroenterology* 2011; **141**: 2210-2217.e2 [PMID: 21875495 DOI: 10.1053/j.gastro.2011.08.033]

10 **Sah RP**, Dudeja V, Dawra RK, Saluja AK. Cerulein-induced chronic pancreatitis does not require intra-acinar activation of trypsinogen in mice. *Gastroenterology* 2013; **144**: 1076-1085.e2 [PMID: 23354015 DOI: 10.1053/j.gastro.2013.01.041]

11 **Kereszturi E**, Szmola R, Kukor Z, Simon P, Weiss FU, Lerch MM, Sahin-Tóth M. Hereditary pancreatitis caused by mutation-induced misfolding of human cationic trypsinogen: a novel disease mechanism. *Hum Mutat* 2009; **30**: 575-582 [PMID: 19191323 DOI: 10.1002/humu.20853]

12 **Logsdon CD**, Ji B. The role of protein synthesis and digestive enzymes in acinar cell injury. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 362-370 [PMID: 23507798 DOI: 10.1038/nrgastro.2013.36]

13 **Waldron RT,** Pandol S, Lugea A, Groblewski G. Endoplasmic Reticulum Stress and the Unfolded Protein Response in Exocrine Pancreas Physiology and Pancretitis. Pancreapedia: Exocrine Pancreas Knowledge Base, 2015 [DOI: 10.3998/panc.2015.41]

14 **Lugea A**, Waldron RT, Pandol SJ. Pancreatic adaptive responses in alcohol abuse: Role of the unfolded protein response. *Pancreatology* 2015; **15**: S1-S5 [PMID: 25736240 DOI: 10.1016/j.pan.2015.01.011]

15 **Sah RP**, Garg P, Saluja AK. Pathogenic mechanisms of acute pancreatitis. *Curr Opin Gastroenterol* 2012; **28**: 507-515 [PMID: 22885948 DOI: 10.1097/MOG.0b013e3283567f52]

16 **Kozutsumi Y**, Segal M, Normington K, Gething MJ, Sambrook J. The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. *Nature* 1988; **332**: 462-464 [PMID: 3352747 DOI: 10.1038/332462a0]

17 **Walter P**, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 2011; **334**: 1081-1086 [PMID: 22116877 DOI: 10.1126/science.1209038]

18 **Ron D**, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 2007; **8**: 519-529 [PMID: 17565364 DOI: 10.1038/nrm2199]

19 **Lugea A**, Gerloff A, Su HY, Xu Z, Go A, Hu C, French SW, Wilson JS, Apte MV, Waldron RT, Pandol SJ. The Combination of Alcohol and Cigarette Smoke Induces Endoplasmic Reticulum Stress and Cell Death in Pancreatic Acinar Cells. *Gastroenterology* 2017; **153**: 1674-1686 [PMID: 28847752 DOI: 10.1053/j.gastro.2017.08.036]

20 **Pandol SJ**, Gorelick FS, Lugea A. Environmental and genetic stressors and the unfolded protein response in exocrine pancreatic function - a hypothesis. *Front Physiol* 2011; **2**: 8 [PMID: 21483727 DOI: 10.3389/fphys.2011.00008]

21 **Harding HP**, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 1999; **397**: 271-274 [PMID: 9930704 DOI: 10.1038/16729]

22 **Harding HP**, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, Ron D. Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol Cell* 2000; **6**: 1099-1108 [PMID: 11106749 DOI: 10.1016/S1097-2765(00)00108-8]

23 **Tsuru A**, Imai Y, Saito M, Kohno K. Novel mechanism of enhancing IRE1α-XBP1 signalling via the PERK-ATF4 pathway. *Sci Rep* 2016; **6**: 24217 [PMID: 27052593 DOI: 10.1038/srep24217]

24 **Marciniak SJ**, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, Nagata K, Harding HP, Ron D. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev* 2004; **18**: 3066-3077 [PMID: 15601821 DOI: 10.1101/gad.1250704]

25 **Wang S**, Kaufman RJ. The impact of the unfolded protein response on human disease. *J Cell Biol* 2012; **197**: 857-867 [PMID: 22733998 DOI: 10.1083/jcb.201110131]

26 **Julier C**, Nicolino M. Wolcott-Rallison syndrome. *Orphanet J Rare Dis* 2010; **5**: 29 [PMID: 21050479 DOI: 10.1186/1750-1172-5-29]

27 **Moreno JA**, Halliday M, Molloy C, Radford H, Verity N, Axten JM, Ortori CA, Willis AE, Fischer PM, Barrett DA, Mallucci GR. Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. *Sci Transl Med* 2013; **5**: 206ra138 [PMID: 24107777 DOI: 10.1126/scitranslmed.3006767]

28 **Maly DJ**, Papa FR. Druggable sensors of the unfolded protein response. *Nat Chem Biol* 2014; **10**: 892-901 [PMID: 25325700 DOI: 10.1038/nchembio.1664]

29 **Iida K**, Li Y, McGrath BC, Frank A, Cavener DR. PERK eIF2 alpha kinase is required to regulate the viability of the exocrine pancreas in mice. *BMC Cell Biol* 2007; **8**: 38 [PMID: 17727724 DOI: 10.1186/1471-2121-8-38]

30 **Gao Y**, Sartori DJ, Li C, Yu QC, Kushner JA, Simon MC, Diehl JA. PERK is required in the adult pancreas and is essential for maintenance of glucose homeostasis. *Mol Cell Biol* 2012; **32**: 5129-5139 [PMID: 23071091 DOI: 10.1128/MCB.01009-12]

31 **Weng TI**, Wu HY, Chen BL, Jhuang JY, Huang KH, Chiang CK, Liu SH. C/EBP homologous protein deficiency aggravates acute pancreatitis and associated lung injury. *World J Gastroenterol* 2013; **19**: 7097-7105 [PMID: 24222953 DOI: 10.3748/wjg.v19.i41.7097]

32 **Waldron RT,** Javaherizadeh P, Gerloff A, Zeng Q, Patterson JB, Pandol SJ, Lugea A. Differential modulation of XBP1 and PERK ER stress pathways in acute pancreatitis. *Pancreatology* 2013; **13**: e83 [DOI: 10.1016/j.pan.2012.12.344]

33 **Lugea A**, Tischler D, Nguyen J, Gong J, Gukovsky I, French SW, Gorelick FS, Pandol SJ. Adaptive unfolded protein response attenuates alcohol-induced pancreatic damage. *Gastroenterology* 2011; **140**: 987-997 [PMID: 21111739 DOI: 10.1053/j.gastro.2010.11.038]

34 **Calfon M**, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, Clark SG, Ron D. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 2002; **415**: 92-96 [PMID: 11780124 DOI: 10.1038/415092a]

35 **Yoshida H**, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 2001; **107**: 881-891 [PMID: 11779464 DOI: 10.1016/S0092-8674(01)00611-0]

36 **Hu P**, Han Z, Couvillon AD, Kaufman RJ, Exton JH. Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1alpha-mediated NF-kappaB activation and down-regulation of TRAF2 expression. *Mol Cell Biol* 2006; **26**: 3071-3084 [PMID: 16581782 DOI: 10.1128/MCB.26.8.3071-3084.2006]

37 **Hetz C**, Thielen P, Matus S, Nassif M, Court F, Kiffin R, Martinez G, Cuervo AM, Brown RH, Glimcher LH. XBP-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy. *Genes Dev* 2009; **23**: 2294-2306 [PMID: 19762508 DOI: 10.1101/gad.1830709]

38 **Vidal RL**, Figueroa A, Court FA, Thielen P, Molina C, Wirth C, Caballero B, Kiffin R, Segura-Aguilar J, Cuervo AM, Glimcher LH, Hetz C. Targeting the UPR transcription factor XBP1 protects against Huntington's disease through the regulation of FoxO1 and autophagy. *Hum Mol Genet* 2012; **21**: 2245-2262 [PMID: 22337954 DOI: 10.1093/hmg/dds040]

39 **Casas-Tinto S**, Zhang Y, Sanchez-Garcia J, Gomez-Velazquez M,

Rincon-Limas DE, Fernandez-Funez P. The ER stress factor XBP1s prevents amyloid-beta neurotoxicity. *Hum Mol Genet* 2011; **20**: 2144-2160 [PMID: 21389082 DOI: 10.1093/hmg/ddr100]

40 **Sado M**, Yamasaki Y, Iwanaga T, Onaka Y, Ibuki T, Nishihara S, Mizuguchi H, Momota H, Kishibuchi R, Hashimoto T, Wada D, Kitagawa H, Watanabe TK. Protective effect against Parkinson's disease-related insults through the activation of XBP1. *Brain Res* 2009; **1257**: 16-24 [PMID: 19135031 DOI: 10.1016/j.brainres.2008.11.104]

41 **Hetz C**, Lee AH, Gonzalez-Romero D, Thielen P, Castilla J, Soto C, Glimcher LH. Unfolded protein response transcription factor XBP-1 does not influence prion replication or pathogenesis. *Proc Natl Acad Sci USA* 2008; **105**: 757-762 [PMID: 18178615 DOI: 10.1073/pnas.0711094105]

42 **Bertolotti A**, Wang X, Novoa I, Jungreis R, Schlessinger K, Cho JH, West AB, Ron D. Increased sensitivity to dextran sodium sulfate colitis in IRE1beta-deficient mice. *J Clin Invest* 2001; **107**: 585-593 [PMID: 11238559 DOI: 10.1172/JCI11476]

43 **Kim RS**, Hasegawa D, Goossens N, Tsuchida T, Athwal V, Sun X, Robinson CL, Bhattacharya D, Chou HI, Zhang DY, Fuchs BC, Lee Y, Hoshida Y, Friedman SL. The XBP1 Arm of the Unfolded Protein Response Induces Fibrogenic Activity in Hepatic Stellate Cells Through Autophagy. *Sci Rep* 2016; **6**: 39342 [PMID: 27996033 DOI: 10.1038/srep39342]

44 **Lee AH**, Scapa EF, Cohen DE, Glimcher LH. Regulation of hepatic lipogenesis by the transcription factor XBP1. *Science* 2008; **320**: 1492-1496 [PMID: 18556558 DOI: 10.1126/science.1158042]

45 **Ozcan U**, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Görgün C, Glimcher LH, Hotamisligil GS. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 2004; **306**: 457-461 [PMID: 15486293 DOI: 10.1126/science.1103160]

46 **Jiang D**, Niwa M, Koong AC. Targeting the IRE1α-XBP1 branch of the unfolded protein response in human diseases. *Semin Cancer Biol* 2015; **33**: 48-56 [PMID: 25986851 DOI: 10.1016/j.semcancer.2015.04.010]

47 **Yuan L**, Li X, Feng J, Yin C, Yuan F, Wang X. IRE1α is essential for Xenopus pancreas development. *J Biomed Res* 2014; **28**: 123-131 [PMID: 24683410 DOI: 10.7555/JBR.28.20130076]

48 **Lee AH**, Chu GC, Iwakoshi NN, Glimcher LH. XBP-1 is required for biogenesis of cellular secretory machinery of exocrine glands. *EMBO J* 2005; **24**: 4368-4380 [PMID: 16362047 DOI: 10.1038/sj.emboj.7600903]

49 **Iwawaki T**, Akai R, Kohno K. IRE1α disruption causes histological abnormality of exocrine tissues, increase of blood glucose level, and decrease of serum immunoglobulin level. *PLoS One* 2010; **5**: e13052 [PMID: 20885949 DOI: 10.1371/journal.pone.0013052]

50 **Kubisch CH**, Sans MD, Arumugam T, Ernst SA, Williams JA, Logsdon CD. Early activation of endoplasmic reticulum stress is associated with arginine-induced acute pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2006; **291**: G238-G245 [PMID: 16574987 DOI: 10.1152/ajpgi.00471.2005]

51 **Cross BC**, Bond PJ, Sadowski PG, Jha BK, Zak J, Goodman JM, Silverman RH, Neubert TA, Baxendale IR, Ron D, Harding HP. The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule. *Proc Natl Acad Sci USA* 2012; **109**: E869-E878 [PMID: 22315414 DOI: 10.1073/pnas.1115623109]

52 **Sah RP**, Garg SK, Dixit AK, Dudeja V, Dawra RK, Saluja AK. Endoplasmic reticulum stress is chronically activated in chronic pancreatitis. *J Biol Chem* 2014; **289**: 27551-27561 [PMID: 25077966 DOI: 10.1074/jbc.M113.528174]

53 **Haze K**, Yoshida H, Yanagi H, Yura T, Mori K. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol Biol Cell* 1999; **10**: 3787-3799 [PMID: 10564271 DOI: 10.1091/mbc.10.11.3787]

54 **Ye J**, Rawson RB, Komuro R, Chen X, Davé UP, Prywes R, Brown MS, Goldstein JL. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol Cell* 2000; **6**: 1355-1364 [PMID: 11163209 DOI: 10.1016/S1097-2765(00)00133-7]

55 **Wang Y**, Vera L, Fischer WH, Montminy M. The CREB coactivator CRTC2 links hepatic ER stress and fasting gluconeogenesis. *Nature* 2009; **460**: 534-537 [PMID: 19543265 DOI: 10.1038/nature08111]

56 **Yamamoto K**, Takahara K, Oyadomari S, Okada T, Sato T, Harada A, Mori K. Induction of liver steatosis and lipid droplet formation in ATF6alpha-knockout mice burdened with pharmacological endoplasmic reticulum stress. *Mol Biol Cell* 2010; **21**: 2975-2986 [PMID: 20631254 DOI: 10.1091/mbc.E09-02-0133]

57 **Naranjo JR**, Zhang H, Villar D, González P, Dopazo XM, Morón-Oset J, Higueras E, Oliveros JC, Arrabal MD, Prieto A, Cercós P, González T, De la Cruz A, Casado-Vela J, Rábano A, Valenzuela C, Gutierrez-Rodriguez M, Li JY, Mellström B. Activating transcription factor 6 derepression mediates neuroprotection in Huntington disease. *J Clin Invest* 2016; **126**: 627-638 [PMID: 26752648 DOI: 10.1172/JCI82670]

58 **Kohl S**, Zobor D, Chiang WC, Weisschuh N, Staller J, Gonzalez Menendez I, Chang S, Beck SC, Garcia Garrido M, Sothilingam V, Seeliger MW, Stanzial F, Benedicenti F, Inzana F, Héon E, Vincent A, Beis J, Strom TM, Rudolph G, Roosing S, Hollander AI, Cremers FP, Lopez I, Ren H, Moore AT, Webster AR, Michaelides M, Koenekoop RK, Zrenner E, Kaufman RJ, Tsang SH, Wissinger B, Lin JH. Mutations in the unfolded protein response regulator ATF6 cause the cone dysfunction disorder achromatopsia. *Nat Genet* 2015; **47**: 757-765 [PMID: 26029869 DOI: 10.1038/ng.3319]

59 **Hawkins JL**, Robbins MD, Warren LC, Xia D, Petras SF, Valentine JJ, Varghese AH, Wang IK, Subashi TA, Shelly LD, Hay BA, Landschulz KT, Geoghegan KF, Harwood HJ Jr. Pharmacologic inhibition of site 1 protease activity inhibits sterol regulatory element-binding protein processing and reduces lipogenic enzyme gene expression and lipid synthesis in cultured cells and experimental animals. *J Pharmacol Exp Ther* 2008; **326**: 801-808 [PMID: 18577702 DOI: 10.1124/jpet.108.139626]

60 **Urata S**, Yun N, Pasquato A, Paessler S, Kunz S, de la Torre JC. Antiviral activity of a small-molecule inhibitor of arenavirus glycoprotein processing by the cellular site 1 protease. *J Virol* 2011; **85**: 795-803 [PMID: 21068251 DOI: 10.1128/JVI.02019-10]

61 **Ren Z**, Wang X, Xu M, Yang F, Frank JA, Ke ZJ, Luo J. Binge ethanol exposure causes endoplasmic reticulum stress, oxidative stress and tissue injury in the pancreas. *Oncotarget* 2016; **7**: 54303-54316 [PMID: 27527870 DOI: 10.18632/oncotarget.11103]

62 **Barrera K,** Okochi K, Stanek A, Mueller C, Ou P, Alfonso A, Huan C. Site-1-Protease mediated unfolded protein response protects against pancreatic injury in acute pancreatitis. *Gastroenterology* 2017; **152**: S894-895 [DOI: 10.1016/S0016-5085(17)33056-1]

63 **Zhang K**, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. *Nature* 2008; **454**: 455-462 [PMID: 18650916 DOI: 10.1038/nature07203]

64 **Yamazaki H**, Hiramatsu N, Hayakawa K, Tagawa Y, Okamura M, Ogata R, Huang T, Nakajima S, Yao J, Paton AW, Paton JC, Kitamura M. Activation of the Akt-NF-kappaB pathway by subtilase cytotoxin through the ATF6 branch of the unfolded protein response. *J Immunol* 2009; **183**: 1480-1487 [PMID: 19561103 DOI: 10.4049/jimmunol.0900017]

65 **Gukovsky I**, Gukovskaya A. Nuclear factor-κB in pancreatitis: Jack-of-all-trades, but which one is more important? *Gastroenterology* 2013; **144**: 26-29 [PMID: 23164573 DOI: 10.1053/j.gastro.2012.11.016]

66 **Algül H**, Treiber M, Lesina M, Nakhai H, Saur D, Geisler F, Pfeifer A, Paxian S, Schmid RM. Pancreas-specific RelA/p65 truncation increases susceptibility of acini to inflammation-associated cell death following cerulein pancreatitis. *J Clin Invest* 2007; **117**: 1490-1501 [PMID: 17525802 DOI: 10.1172/JCI29882]

67 **Neuhöfer P**, Liang S, Einwächter H, Schwerdtfeger C, Wartmann T, Treiber M, Zhang H, Schulz HU, Dlubatz K, Lesina M, Diakopoulos KN, Wörmann S, Halangk W, Witt H, Schmid RM, Algül H. Deletion of IκBα activates RelA to reduce acute pancreatitis in mice through up-regulation of Spi2A. *Gastroenterology* 2013; **144**: 192-201 [PMID: 23041330 DOI: 10.1053/j.gastro.2012.09.058]

68 **Baumann B**, Wagner M, Aleksic T, von Wichert G, Weber CK, Adler G, Wirth T. Constitutive IKK2 activation in acinar cells is sufficient to induce pancreatitis in vivo. *J Clin Invest* 2007; **117**: 1502-1513 [PMID:

17525799 DOI: 10.1172/JCI30876]
69 **Huang H**, Liu Y, Daniluk J, Gaiser S, Chu J, Wang H, Li ZS, Logsdon CD, Ji B. Activation of nuclear factor-κB in acinar cells increases the severity of pancreatitis in mice. *Gastroenterology* 2013; **144**: 202-210 [PMID: 23041324 DOI: 10.1053/j.gastro.2012.09.059]
70 **Tam AB**, Mercado EL, Hoffmann A, Niwa M. ER stress activates NF-κB by integrating functions of basal IKK activity, IRE1 and PERK. *PLoS One* 2012; **7**: e45078 [PMID: 23110043 DOI: 10.1371/journal.pone.0045078]
71 **Xu P**, Wang J, Yang ZW, Lou XL, Chen C. Regulatory roles of the PI3K/Akt signaling pathway in rats with severe acute pancreatitis. *PLoS One* 2013; **8**: e81767 [PMID: 24312352 DOI: 10.1371/journal.pone.0081767]
72 **Gukovskaya AS**, Gukovsky I. Autophagy and pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2012; **303**: G993-G1003 [PMID: 22961802 DOI: 10.1152/ajpgi.00122.2012]
73 **Yan D**, Wang HW, Bowman RL, Joyce JA. STAT3 and STAT6 Signaling Pathways Synergize to Promote Cathepsin Secretion from Macrophages via IRE1α Activation. *Cell Rep* 2016; **16**: 2914-2927 [PMID: 27626662 DOI: 10.1016/j.celrep.2016.08.035]
74 **Zhou Y**, Lee J, Reno CM, Sun C, Park SW, Chung J, Lee J, Fisher SJ, White MF, Biddinger SB, Ozcan U. Regulation of glucose homeostasis through a XBP-1-FoxO1 interaction. *Nat Med* 2011; **17**: 356-365 [PMID: 21317886 DOI: 10.1038/nm.2293]
75 **Li N**, Wu X, Holzer RG, Lee JH, Todoric J, Park EJ, Ogata H, Gukovskaya AS, Gukovsky I, Pizzo DP, VandenBerg S, Tarin D, Atay C, Arkan MC, Deerinck TJ, Moscat J, Diaz-Meco M, Dawson D, Erkan M, Kleeff J, Karin M. Loss of acinar cell IKKα triggers spontaneous pancreatitis in mice. *J Clin Invest* 2013; **123**: 2231-2243 [PMID: 23563314 DOI: 10.1172/JCI64498]
76 **B'chir W**, Maurin AC, Carraro V, Averous J, Jousse C, Muranishi Y, Parry L, Stepien G, Fafournoux P, Bruhat A. The eIF2α/ATF4 pathway is essential for stress-induced autophagy gene expression. *Nucleic Acids Res* 2013; **41**: 7683-7699 [PMID: 23804767 DOI: 10.1093/nar/gkt563]
77 **Zalckvar E**, Berissi H, Mizrachy L, Idelchuk Y, Koren I, Eisenstein M, Sabanay H, Pinkas-Kramarski R, Kimchi A. DAP-kinase-mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-XL and induction of autophagy. *EMBO Rep* 2009; **10**: 285-292 [PMID: 19180116 DOI: 10.1038/embor.2008.246]
78 **Solakoglu T**, Akyol P, Guney T, Dilek I, Atalay R, Koseoglu H, Akin E, Demirezer Bolat A, Buyukasik NS, Ersoy O. Acute pancreatitis caused by bortezomib. *Pancreatology* 2013; **13**: 189-190 [PMID: 23561979 DOI: 10.1016/j.pan.2013.01.002]
79 **O'Keefe SJ**, Lee RB, Anderson FP, Gennings C, Abou-Assi S, Clore J, Heuman D, Chey W. Physiological effects of enteral and parenteral feeding on pancreaticobiliary secretion in humans. *Am J Physiol Gastrointest Liver Physiol* 2003; **284**: G27-G36 [PMID: 12488233 DOI: 10.1152/ajpgi.00155.2002]
80 **van Santvoort HC**, Besselink MG, Bakker OJ, Hofker HS, Boermeester MA, Dejong CH, van Goor H, Schaapherder AF, van Eijck CH, Bollen TL, van Ramshorst B, Nieuwenhuijs VB, Timmer R, Laméris JS, Kruyt PM, Manusama ER, van der Harst E, van der Schelling GP, Karsten T, Hesselink EJ, van Laarhoven CJ, Rosman C, Bosscha K, de Wit RJ, Houdijk AP, van Leeuwen MS, Buskens E, Gooszen HG; Dutch Pancreatitis Study Group. A step-up approach or open necrosectomy for necrotizing pancreatitis. *N Engl J Med* 2010; **362**: 1491-1502 [PMID: 20410514 DOI: 10.1056/NEJMoa0908821]

P- Reviewer: Mann O, Nagahara H, Zhu X, Zhu X **S- Editor**: Cui LJ
L- Editor: Filipodia **E- Editor**: Yin SY

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: http://www.f6publishing.com

World J Gastrointest Pathophysiol 2018 September 29; 9(2): 47-58

DOI: 10.4291/wjgp.v9.i2.47

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Basic Study

Gut microbiome profiling and colorectal cancer in African Americans and Caucasian Americans

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Supported by Department of Veteran Affairs, No. 1I101BX001927; National Institutes of Health, No. 1R21CA175916.

Institutional review board statement: The study followed a Full Board Review by the Wayne State University Institutional Review Board.

Informed consent statement: All routine colonoscopy, collection of colonic effluents and biopsy specimens from the patients were performed after obtaining informed consent and ethical permission.

Conflict-of-interest statement: To the best of our knowledge, no conflict of interest exists.

ARRIVE guidelines statement: The ARRIVE Guidelines had been adopted.

Manuscript source: Unsolicited Manuscript

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Received: May 11, 2018
Peer-review started: May 11, 2018
First decision: July 10, 2018
Revised: August 8, 2018
Accepted: August 26, 2018
Article in press: August 26, 2018
Published online: September 29, 2018

Abstract

AIM

To determine whether and to what extent the gut microbiome is involved in regulating racial disparity in colorectal cancer (CRC).

METHODS

All patients were recruited and experiments were performed in accordance with the relevant guidelines and regulations by the Institutional Review Boards (IRB), committees of the John D. Dingell VAMC and Wayne State University guidelines. African American (AA) and Caucasian American (CA) patients were scheduled for an outpatient screening for colonoscopy, and no active malignancy volunteer patients were doubly consented, initially by the gastroenterologist and later by the study coordinator, for participation in the study. The gut microbial communities in colonic effluents from AAs and CAs were examined using 16sRNA profiling, and bacterial identifications were validated by performing SYBR-based Real Time PCR. For metagenomic analysis to characterize the microbial communities, multiple software/tools were used, including Metastats and R statistical software.

RESULTS

It is generally accepted that the incidence and mortality of CRC is higher in AAs than in CAs. However, the reason for this disparity is not well understood. We hypothesize that the gut microbiome plays a role in regulating this disparity. Indeed, we found significant differences in species richness and diversity between AAs and CAs. *Bacteroidetes* was more abundant in AAs than in CAs. In particular, the pro-inflammatory bacteria *Fusobacterium nucleatum* and *Enterobacter* species were significantly higher in AAs, whereas probiotic *Akkermansia muciniphila* and *Bifidobacterium* were higher in CAs. The polyphyletic *Clostridia* class showed a divergent pattern, with *Clostridium XI* elevated in AAs, and *Clostridium IV*, known for its beneficial function, higher in CAs. Lastly, the AA group had decreased microbial diversity overall in comparison to the CA group. In summary, there were significant differences in pro-inflammatory bacteria and microbial diversity between AA and CA, which may help explain the CRC disparity between groups.

CONCLUSION

Our current investigation, for the first time, demonstrates microbial dysbiosis between AAs and CAs, which could contribute to the racial disparity of CRC.

Key words: Human gut; Microbiome; Colorectal cancer; *Fusobacterium nucleatum*; African Americans; 16S RNA profiling; Metagenomics

Core tip: Several studies have demonstrated that the incidence of colorectal cancer (CRC) is higher in African Americans than Caucasian Americans. Reasons for this racial disparity are unknown. The current study, for the first time, demonstrated that dysbiosis in the gut microbiome plays a determinant role in the racial disparity of CRC. Determining the influence of the microbiota on the risk of developing CRC will have a major impact on health, since early-stage CRC hinges on the ability to detect early pathological changes. Subsequent translational studies could also be developed to alter microbiota with medications or diet, thus reducing the risk of developing CRC.

Farhana L, Antaki F, Murshed F, Mahmud H, Judd SL, Nangia-Makker P, Levi E, Yu Y, Majumdar APN. Gut microbiome profiling and colorectal cancer in African Americans and Caucasian Americans. *World J Gastrointest Pathophysiol* 2018; 9(2): 47-58 Available from: URL: http://www.wjgnet.com/2150-5330/full/v9/i2/47.htm DOI: http://dx.doi.org/10.4291/wjgp.v9.i2.47

INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of cancer death in the United States, and several studies have demonstrated that African Americans (AAs) have the highest rate of CRC in the United States[1-6]. AAs have the highest incidence and death rates for CRC than Caucasian Americans (CAs), Hispanics and Asian/Pacific Islanders[7]. CRC typically correlates with age, reflecting a multistep progression from normal epithelium to carcinoma. However, a significant number of AAs are diagnosed with CRC at a younger age compared to CAs[8-11]. Genomic alterations in oncogenes and tumor suppressor genes drive the epithelial cell transformation to carcinoma - including the Apc/Wnt-β-catenin signaling pathway and the tumor suppressor gene Apc[12-14]. Previous studies have shown that microRNA drivers upregulated in AAs lead to an increased proportion of cancer stem cells in human colonic epithelial cells[15].

The human microbiome is at the interface of intrinsic and environmental factors - and abnormalities in the gut microbiome have been noted in patients with CRC[16-19]. The colonic microbiota is mostly bacteria consisting of approximately 10^3 different microbial species[20]. Gut microbiota is essential in the maintenance of homeostasis, and it contributes to immune development, inhibits pathogen colonization, processes drug metabolites, metabolizes nutrients from the diet and also modulates their biological activities[10,21]. Dysregulation of gut microbiota and a concomitant state of chronic inflammation and persistent activation of the host immune system have been implicated in the initiation and development of CRC[22-24].

One primary role of gut bacteria is to participate in the biotransformation of products in the gut, which include bile acids secreted from the liver. The gut microbiota may alter cancer susceptibility and has anti-cancer effect through the production of microbial secretory

Table 1 General characteristics of African American and Caucasian American patients

Race	Gender	Age	Height (inches)	Weight (lbs)	Body mass index	Polyp	Adenoma
AA	Male	65.2	70.4	181	28.9	4.6	3.8
CA	Male	62.6	69.0	194	31.0	1.43	1.0

AA: African Americans; CA: Caucasian Americans.

metabolites, such as short chain fatty acids (SCFAs), secondary bile acids (SBA), and trimethylamine N-oxide (TAMO)[25-28]. SBAs, deoxycholic acid (DCA) and lithocholic acid (LCA), are noted in particular for their carcinogenic activity[29-33]. Murine models have demonstrated that DCA shifts the microbiota community to dysbiosis and promotes intestinal tumorigenesis in $Apc^{min/+}$ mice when DCA-treated fecal microbiota inoculated in one mouse is transferred to another[34].

Thus, multifactorial reasons underlie the racial disparity of CRC. The current investigation was aimed at studying microbial dysbiosis in the gut between AAs and CAs. In this pilot study, we investigate the diversity and abundance of specific gut microbes in colonic effluents using 16S rRNA gene community profiles in AAs and CAs and their possible role in increased incidence of CRC in AAs.

MATERIALS AND METHODS

Study subjects and collection of samples

In the current pilot investigation, 52 AA and 46 CA patients were recruited. The study was approved by the Institutional Review Boards and Committees of the John D. Dingell-Veterans Affairs Medical Center (JDD-VAMC) and Wayne State University School of Medicine. Patients excluded from the study were those with active malignant disease, inflammatory bowel disease, recent infection and those recently treated with antibiotics. In addition, patients with psychiatric or addictive disorder, hemorrhagic diathesis or on warfarin were excluded. General characteristics of study participants are the same as described in our earlier publication[15]. None of the patients were taking probiotics as supplements. General characteristics of each group of patients are presented in Table 1.

Eligible study subjects were scheduled for an outpatient screening colonoscopy at the JDD-VAMC. All study subjects received standard colonoscopy purgative preparation. Briefly, patients were asked to stay on a clear liquid diet for 24 h and to take a preparation containing 15 mg Bisacodyl the morning prior to their colonoscopy. The patients were also instructed to split the dose (4 L) of poly-ethylene glycol solution (PEG) into a first half (2 L) the evening prior to colonoscopy, and to drink the second half (the remaining 2 L) 5 h prior to the procedure and to finish it 3 h prior to the procedure, regardless of appointment time (morning or afternoon). Colonic effluent was aspirated prior to the colonoscopy through the working channel of the endoscope, as reported earlier[15]. Additionally, 8 forceps biopsies were taken from macroscopically normal appearing colonic mucosa (< 10 cm anal verge), as described previously[15].

DNA extraction for 16S rRNA gene microbial community profiling

Genomic DNA was extracted from colonic effluents using QIAamp DNA Stool Mini Kit (QIAGEN) according to the manufacturer's instructions. Purified DNA used for analysis of 16S rRNA community profiling was performed by LC Sciences (Houston, Texas). The V3 and V4 regions of the 16S rRNA gene were amplified to generate approximately 469 bp amplicons, automating cluster generation and sequencing on the MiSeq system. For data analysis, the merge paired-end reads from DNA fragments were analyzed using next generation sequencing FLASH (Fast Length Adjustment SHort reads) software, and raw sequencing data quality control was checked using FastQC software. Operational taxonomic units (OTUs) clustering was based on 97% sequence similarity using CD-HIT software. Microbial strain identification software Quantitative Insights Into Microbial Ecology (QIIME) was used for alpha diversity and beta diversity, visualization of high throughput microbial community, and for principal coordinates analysis. Ribosomal Database Project (RDP) classifier, Greengenes, NCBI 16SMicrobial (TUIT tool) and GraPhlAn software were used for taxonomic classification and circular taxonomic phylogenetic trees.

Genomic DNA isolation from colonic effluents and validation

Bacterial genomic DNA was isolated from colonic effluents using QIAamp DNA Stool Mini Kit (QIAGEN) according to the manufacturer's instructions. For real time PCR, DNA (40-50 ng) and appropriate blank were used for RT-PCR analysis in triplicate using the 2 × Power-Up SYBR Green PCR Master Mix (Applied Biosystems) and the ABI Prism 7500 sequence detection system. PCR consisted of 40 cycles of 95 ℃ for 10 min and then 95 ℃ for 15 s, 60 ℃ for 60 s. The primer sequences were used to evaluate the presence of specific types of bacteria. Ct values were utilized to assess the relative concentration of specific DNA for each sample as described by the manufacturer. Each sample $^{\Delta\Delta}$Ct values was calculated by normalizing to the CT value of total bacteria (Eubacteria). 16S rDNA served as an internal control and each value represented the mean of three replicates. All oligonucleotide primers were synthesized by Integrated DNA technology Inc. (Coralville, IA, United States). The primer set for each

Table 2 List of primers for bacterial genes specific for family, genus and species

Bacterial gene	Forward primer (5'-3')	Reverse primer (5'-3')	Ref.
Clostridium cluster XI	TGACGGTACYYNRKGAGGAAGCC	CTACGGTTRAGCCGTAGCCTTT	[63]
Clostridium cluster XIVa	GCGGTRCGGCAAGTCTGA	CCTCCGACACTCTAGTMCGAC	[64]
Clostridium cluster IV	GCACAAGCAGTGGAGT	CTTCCTCCGTTTTGTCAA	[65]
Bifidobacterium genus	GATTCTGGCTCAGGATGAACGC	CTGATAGGACGCGACCCCAT	[66]
Lactobacillus spp.	AGCAGTAGGGAATCTTCCA	CACCGCTACACATGGAG	[66]
Enterobacter (Family)	CATTGACGTTACCCGCAGAAGAAGC	CTCTACGAGACTCAAGCTTGC	[66]
Fusobacterium (genus)	GGATTTATTGGGCGTAAAGC	GGCATTCCTACAAATATCTACGAA	[67]
Fusobacterium nucleatum	CAACCATTACTTTAACTCTACCATGTTCA	GTTGACTTTACAGAAGGAGATTATGTAAAAATC	[68]
Clostridium sordelli	CTGAGACACGTCCAAACTCTAC	CCTCCTCAAGTACCGTCATTATC	-
Total bacteria	CGTGCCAGCAGCCGCGG	TGGACTACCAGGGTATCTAATCCTG	

gene is listed in Table 2.

Real-Time PCR from biopsy

To determine the specific bacterial abundance between serrated and tubular adenomatous patients, total RNA was prepared from patient biopsy samples using TRIzol as recommended by the manufacturer and purified using the Rneasy Mini Kit (QIAGEN). For real time PCR, cDNA was prepared with the SuperScript Ⅲ First-Strand cDNA synthesis system for RT-PCR (Invitrogen) and analyzed in triplicate using the 2 × Power-Up SYBR Green PCR Master Mix (Applied Biosystems) and the ABI Prism 7500 sequence detection system. Primers and PCR were performed as described in the previous section.

For determination of RT-PCR expression of 7-α-dehydroxylase (BaiCD), primers were as follows, *baiCD* forward: 5'-GGWTTCAGCCCRCAGATGTTCTTTG-3'; reverse: 5'GAATTCCGGGTTCATGAACATTCTKCKAAG-3'[35].

Statistical analysis

For microbiota data statistical analysis, Metastats software was used for metagenomics sequencing data analyzed from two groups to characterize the microbial communities. CD-HT and R statistical software was used for BIOM-formatted OTU communities clustering and OTU statistics. For examining alpha diversity, QIIME software was used for graphics and statistical purposes. RDP classifier, QIIME, TUIT GraPhlAn, MetaPhlAn, R software/Too were used for taxonomic classification and statistics. For Real Time PCR data, the standard deviation of mean between two groups and t-test were performed to determine the significance level between two groups.

RESULTS

Phylogenetic analysis of microbial communities in colonic effluents

Microbiota composition of colonic effluent was compared by high throughput analysis of 16S small ribosomal subunit gene (16S rRNA) amplicon. Sequencing of the V3 + V4 region was used for OTU clustering based on 97% sequence similarity. We found unique OTUs in AAs (7234) and CAs (5252), with an overlap of 742 OTUs between the two groups (Figure 1A). We found higher species richness and species diversity in CAs using the number of OTUs in Shannon index (Figure 1B). Irrespective of high inter-individual variances, the Principal Coordinates Analysis (PCoA) showed AAs to possess an abundance of common microbiota, compared to dispersed CA counterparts, revealing more microbial homogeneity within AAs than CA patients (Figure 1C).

The microbiota composition of AAs and CAs showed significant differences in the *Bacteroidetes* and *Proteobacteria* phyla (Figure 2A and Table 3). Taxonomic phylum from 11 from AAs and 24 from CAs were identified (Figure 2A). *Bacteroidetes* was the most abundant bacterial phylum in AAs (70%), whereas *Firmicutes* occurrence of 36% was higher in CAs (Figure 2A). Phylogenetic analysis further identified 44 classes of microbiota, with CAs showing more diverse population than AAs (Figure 2B). *Bacteroidia* was significantly higher in AAs, while *Clostridia, Bacteria-unclassified, Gammaproteobacteria, Bacilli, Verrucomicrobiae, Acinobacteria, Fusobacteria* and *Alphaproteobacteria* were more abundant in CAs (Figure 2B). Again, the microbial species richness and diversity was higher in CAs compared to AAs (Figure 2B).

By 16S rRNA gene profiling, there were eight predominant families found between the two racial groups, *Bacteroidaceae*, *Ruminococcaceae*, *Lachnospiraceae*, *Rikenallaceae, Porphymonadaceae, streptococaceae, Acidaminococaceae and Veillonellaceae* (Table 3). The most predominant genus in AAs and CAs was *Bacteroides*, comprising 56% and 29%, respectively. Genus abundance of other microbiota was less than 7% with some degree of variation, as observed for *Gemmiger, Allistipes, Parabacterroides, Faecalibacterium, Biophila, Ruminococcus2, Escherichia/Shigella (E/S), Streptococcus, Clostridium IV, Clostridium XIVa, Barnesiella, Akkermansia, Phascolartobacterium, Veillonella, Blautica, Roseburia, Haemophilus, Dialister* and *Fusobacterium* (Table 3).

At the species levels, the relative abundance of *B. caccae* and *B. massiliensis, P. distasonis, P. unclassified, Biophila unclassified* and *Clostridium XI unclassified* were noted to be significantly higher in AAs compared to CAs (Table 3). In CA, the abundance of *G. formicilis, Clostridium IV, B. intestinihominis, E/S. unclassified, H. parainfluenza, A. muciniphila, D. invisus, S. faecium* and *F. unclassified* abundance was higher than AA patients.

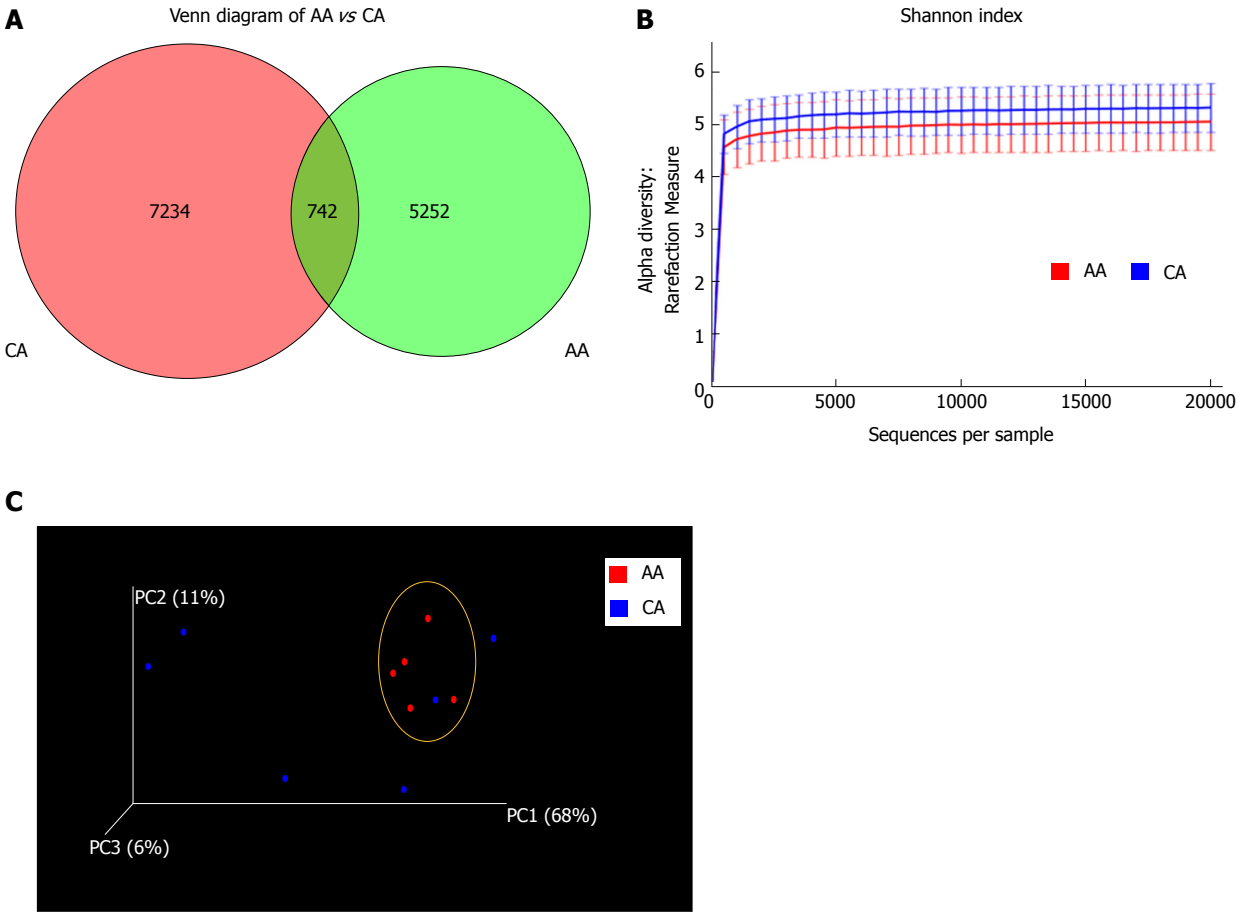

Figure 1 Microbial diversity in colonic effluent from African Americans and Caucasian Americans. A: Venn diagram showing the unique Operational taxonomic units (OTUs) in different subsets of African American (AA) and Caucasian Americans (CA) including overlap community; B: Rarefaction curves showing the species richness from the average number of OTUs for AA and CA (alpha diversity); C: Principal coordinates analysis for beta diversity showing the very dissimilar individual in the CA group while closely similar to the group indicated by the yellow circle. CD-HIT and R software were used for BIOM format OTU clustering and OTU statistics. For measurement of alpha diversity of observed species, QIIME software and Shannon index were used and the emperor tool generated the PCoA plot.

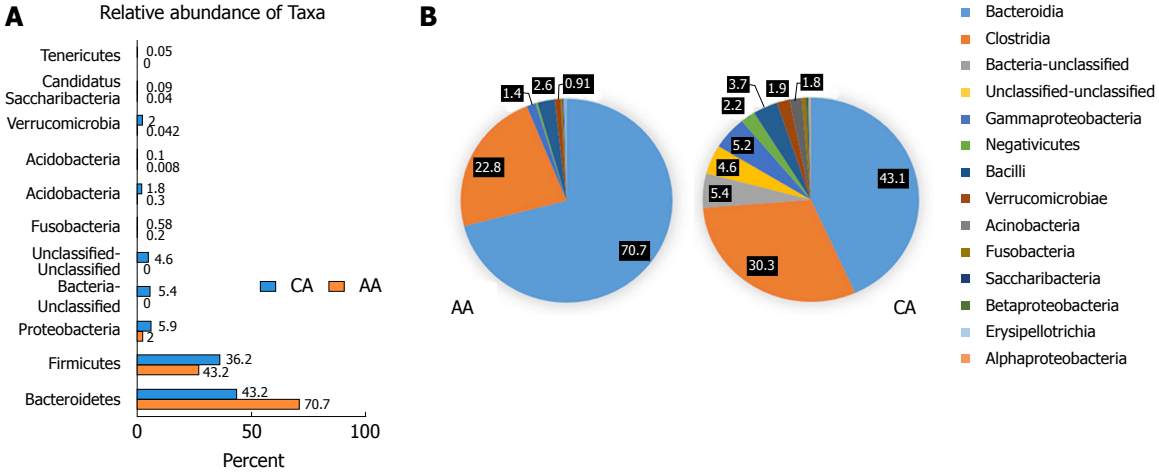

Figure 2 Microbial composition of each group at the phylum and class levels. A: Bar chart shows the relative abundance of phylum; B: Pie chart show the proportion of predominant different classes of microbial community in African Americans and Caucasian Americans. In order to get more comprehensive and accurate taxonomies, multiple databases, Ribosomal Database Project classifier, Greengenes and NCBI 16S Microbial were used for analysis of plot bars and pie charts.

However, with respect to *F. prausnitzii, Ruminococcus2 unclassified, A. putredinis,* and *P. clara*, the relative abundance was found to be similar in both groups (Table 3). On the other hand, the *Fusobacterium* genus was higher in CAs compared to AAs (Figure 3A), and the *Fusobacterium* species level was identified as unclassified by microbial 16sRNA gene profiling.

To further compare the microbial population between

Table 3 Abundance of microbiota in colonic effluents from African Americans and Caucasian Americans (green, black, blue and red color represent phylum, family, genus and species, respectively)

Phylum	Family	Genus	Species	AA (%)	CA (%)
Bacteroidetes				70.74	43.17
	Bacteroidaceae			56.75	29.94
		Bacteroides		56.8	29.9
			Unclassified	30.3	17.8
			Caccae	13.6	5.5
			Massiliensis	6.7	1.7
			Uniformis	2.8	1.5
			Fragilis	1	2
	Rikenellaceae			6.44	5.47
		Allistipes	Putredinis	6.4 (3.9)	5.4 (3.0)
	Porphymonadaceae			6.4	6.95
		Parabacteroides	Distasonis	4.9 (3.4)	3.2 (1.0)
		Barnesiella	Intestinihominis	0.16 (0.16)	2.7 (2.6)
	Prevotellaceae			1.9	1.1
		Paraprevotella	Clara	1.0 (0.9)	0.6 (0.6)
Firmicutes				26.74	36.25
	Ruminococaceae			11.35	15.7
		Faecalibacterium	Prausinitzii	4.5 (4.5)	3.1 (3.1)
		Unclassified	Unclassified	2.5 (2.5)	2.9 (3.0)
		Gemmiger	Formicilis	1.9 (1.6)	6.1 (6.0)
		Clostridium IV	Unclassified	0.3 (0.3)	1.5 (1.5)
	Lachnospiraceae			11.19	13.7
		Ruminococcus 2	Unclassified	3.4 (3.4)	4.7 (4.7)
		Clostridium XIVa	Unclassified	2.05 (2.0)	1.03 (1.0)
		Unclassified	Unclassified	1.6 (1.7)	2.4 (2.4)
		Blautica	Producta	0.22 (0.06)	1.2 (0.0)
		Dorea	Unclassified	1.8 (0.8)	1.3 (1.0)
		Roseburia	Unclassified	0.5 (0.3)	1.3 (0.9)
	Steptococaccae	Steptococcus	Faecium	0.9 (0.9) (0.8)	3.2 (3.2) (3.2)
	Acidaminococcaceae	Phascolarctobacterium	Unclassified	1.8 (1.9) (1.4)	0.9 (0.9) (1.3)
	Peptostreptococcaceae	Clostridium XI	Unclassified	0.02 (0.02) (0.02)	0.01 (0.008) (0.008)
	Veillonellaceae	Veillonella	Atypica	0.81 (0.03) (0.03)	1.27 (0.02) (0.02)
			Unclassified	0.6 (0.6)	0.5 (0.5)
		Dialister	Invisus	0.002 (0.0)	0.68 (0.65)
	Lactobacillaceae	Lactobacillus	Sanfrancisecnsis	0.035 (0.02) (0.004)	0.01 (0.01) (0.001)
Proteobacteria				2.02	5.9
	Desulfovibrionaceae	Biophila	Unclassified	0.16 (0.12) (0.12)	0.07 (0.07) (0.01)
	Sutterrellaceae	Parasuttrella	Excrementihomis	0.50 (0.76) (0.4)	0.24 (1.9) (0.1)
	Pasteurellaceae	Haemophilus	Parainfluenza	0.57 (0.9) (0.4)	1.98 (1.9) (1.9)
	Enterobacteiaceae	Escherichia/Shigella	Unclassified	0.92 (0.8) (0.9)	3.14 (3.1) (3.1)
		Klebsiella	Unclassified	(0.09) (0.02)	0.003 (0.01)
Fusobacteria				0.18	0.58
	Fusobactereaceae	Fusobacterium	Unclassified	0.18 (0.15) (0.15)	0.58 (0.6) (0.6)
Verrucomicrobia				0.04	1.9
	Verrucomicrobiaceae	Akkermansia	Muciniphila	0.04 (0.04)	1.9 (1.9)
Bacteria-unclassified	Unclassified	Unclassified	Unclassified	0	5.4 (5.4) (5.4)
Unclassified-unclassified	Unclassified	Unclassified	Unclassified	0	4.6 (4.6) (4.6)

AA: African Americans; CA: Caucasian Americans.

AAs and CAs, we examined the abundance of specific bacterial populations using species-specific primers. The occurrence of the *Enterobacter* genus was found to be considerably higher in AAs than CAs (Figure 3B), while the *Enterobacteriaceae* family showed the opposite pattern (Table 3). Taxonomic analysis showed CAs to contain *Citrobacter, Klebsiela Escherichia coli, Enterobacter and Shigella sp.* (Table 3). In contrast, the relative abundance of the probiotic bacteria genuses *Bifidobacterium* and *Akkermansia muciniphila* was considerably lower in AAs compared to CAs (Figures 3C and D). These observations demonstrate that the population of pro-inflammatory bacteria is higher in the gut of AAs than CAs.

Occurrence of Fusobacterium nucleatum and Clostridium genus

The relative abundance of *Fusobacterium nucleatum* has been associated with the development and progression of CRC[23,36,37]. We found that the relative abundance of *F. nucleatum* in colonic effluents was significantly higher in AAs than CAs (Figure 3E), indicating a greater risk for the development of CRC in AAs. On the other hand, serrated polyps, which supposedly possess a greater risk of developing CRC, did not exhibit an increased abundancy of *F. nucleatum*. In fact, we found the relative

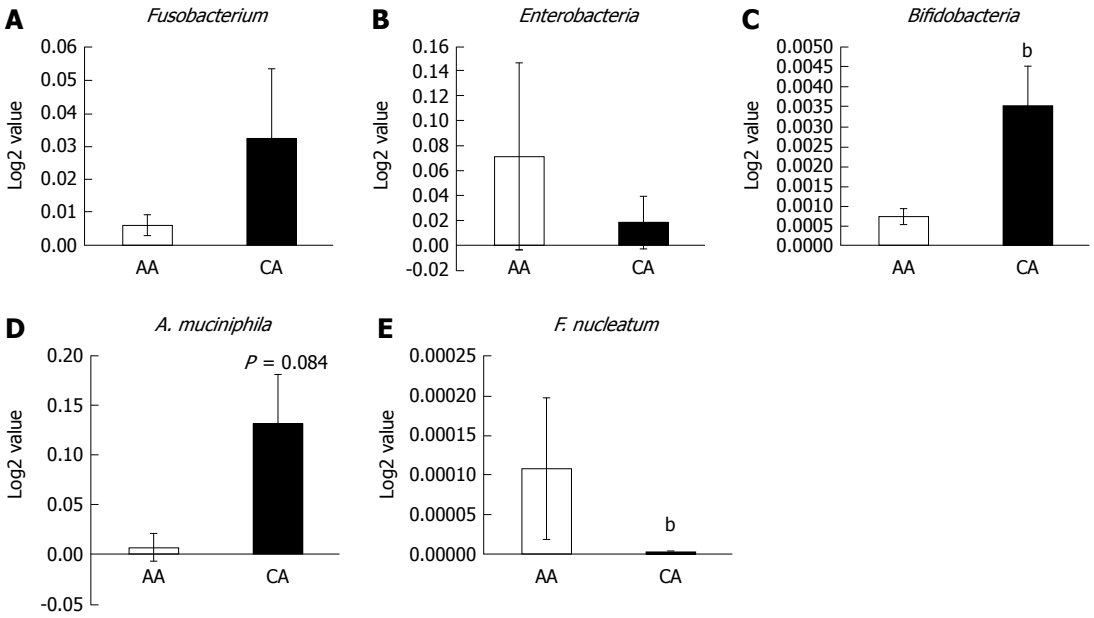

Figure 3 Abundance of inflammatory and probiotic bacteria in colonic effluent from two racial groups using RT-PCR. A: Genus, *Fusobacterium* occurrence higher in Caucasian American (CA); B: The relative abundance of pro-inflammatory *Enterobacter* occurrence is higher in African Americans (AAs); C and D: Probiotic *Bifidobacteria* and *A. muciniphila* is higher in CAs; E: Relative abundance of *F. nucleatum* is higher in AAs than CAs. Data represent mean ± SD of all samples from each group ($^{b}P < 0.001$).

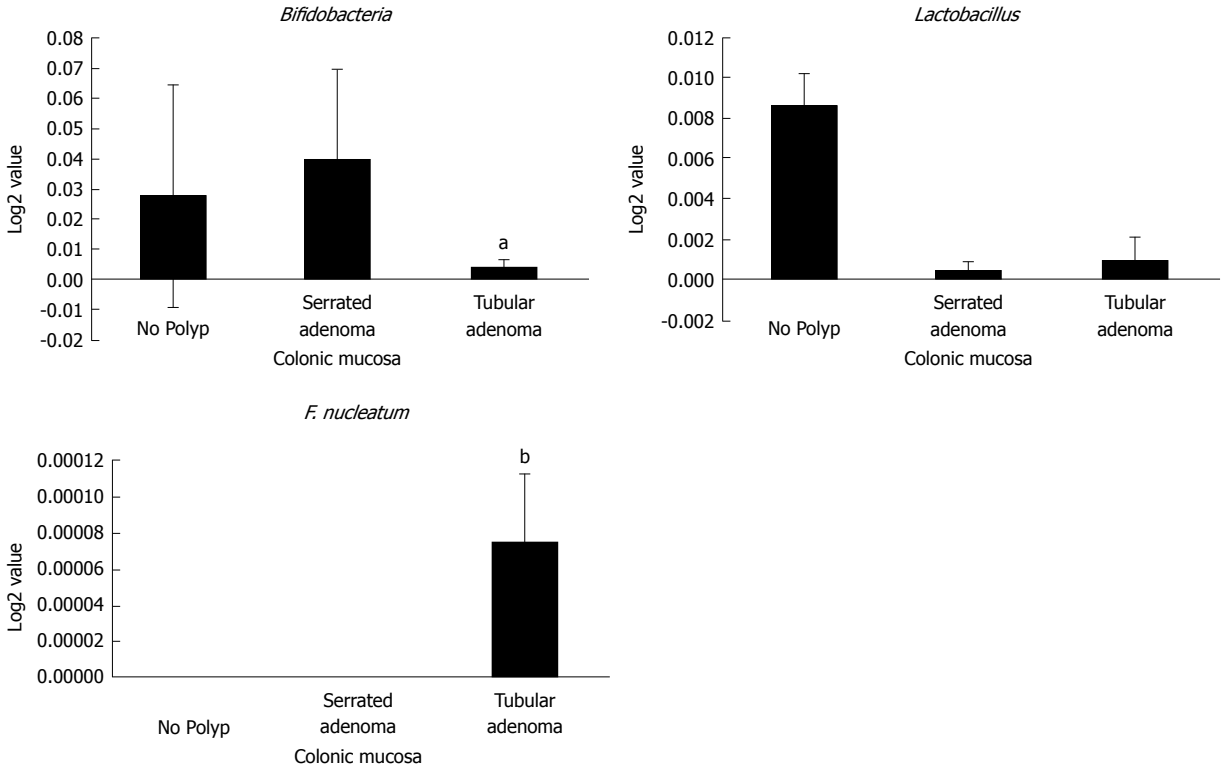

Figure 4 Abundance of probiotic and pro-carcinogenic bacteria in serrated and tubular adenomatous in colonic mucosa. African American and Caucasian American patients were combined. Data represent mean ± SD of all samples from each group ($^{a}P < 0.05$, $^{b}P < 0.001$).

abundance of *F. nucleatum* to be higher in tubular adenoma than serrated adenoma (B-Raf proto oncogene, serine/threonine kinase (BRAF) mutation), whereas the probiotic *Lactobacillus* was lower in both serrated and tubular adenomas than those without adenoma (Figure 4). Likewise, the relative abundance of *Bifidobacteria* was found to be lower in tubular adenoma than those without adenoma (Figure 4).

Using a clostridium cluster analysis and RT-PCR, we found that the relative abundance of *Clostridium IV* was higher in CAs (Figure 5A), while *Clostridium XI* was significantly higher in AAs (Figure 5B). *Clostridium IV*

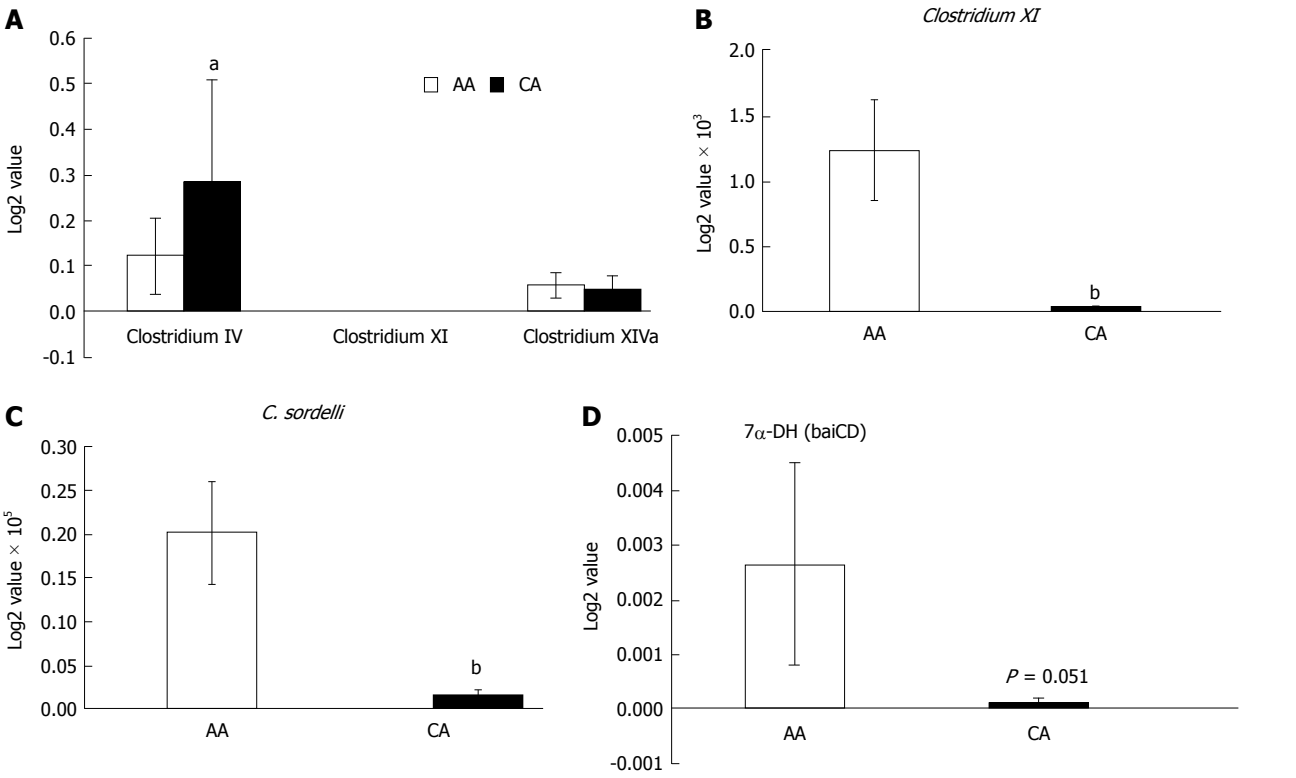

Figure 5 Expression of secondary bile acids transforming enzyme 7-α-DH in the colonic effluent from African Americans and Caucasian Americans using RT-PCR. A: Distribution of different *Clostridium* cluster between African Americans (AA) and Caucasian Americans (CA); B: *Clostridium XI* expression was higher in AAs; C: Secondary bile acids transforming bacteria *Clostridium sordelli* was higher in AAs than CAs; D: Increased 7α-DH expression in AAs. Data represent mean ± SD of all samples from each group ($^{a}P < 0.05$, $^{b}P < 0.01$).

is known to be mediate anti-inflammatory effects[38-40]. *Clostridium sordelli* in the *Clostridium XI* cluster group is known to transform SBA[41]. AA patients also showed higher concentrations of *C. sordelli,* compared to CAs (Figure 5C). A few species of gut anaerobes in the *Clostridium* genus promote the biotransformation of primary to SBA. Given the role of SBA (DCA and LCA) in promoting CRC, the expression of 7-α-dehydroxylase, an enzyme that participates in de-conjugation of primary bile acids, was examined. We found the expression of 7-α-dehydroxylase (baiCD) in colonic effluent from AA patients to be markedly higher than CA subjects (Figure 5D).

DISCUSSION

Our pilot study comparing AA and CA gut microbiota from colonic effluents reveal three major differences between the groups: the AA gut microbiota was less diverse overall, AAs had more pro-inflammatory gut bacteria, and AAs had fewer anti-inflammatory gut bacteria. The phylogenetic tree of microbiota between AAs and CAs reveal an abundance of taxon in CA *vs* AA (Figure 6). Analyzing PCoA of patients, we noted trends found previously in comparing normal colon to adenomas and CRC[36,42,43]. Patients with colonic adenoma typically demonstrate reduced species richness and diversity compared to those without adenomas[44,45]. Furthermore, a fecal microbiota shift occurs in patients with adenomas[46], and they exhibit increased diversity in mucosa than those without adenoma[47].

The human gut microbiota composition is generally represented by three primary phyla: *Firmicutes* (30%-50%), *Bacteroidetes* (20%-40%), and *Actinobacteria* (1%-10%)[22]. The two predominant bacterial phyla, *Bacteroidetes*, and *Firmicutes,* which contribute to 95% of the total GI ecosystem, are associated with adenomas and CRC[48]. The abundance of phylum *Bacteroidetes (P. distasonis, Alistipes spp.)* in the gut may increase the rate of tumorigenesis[49]. Our current study demonstrates an abundance of *Bacteroidetes* (70%) and reduction in *Firmicute* and *Actinobacteria* in AAs, whereas the microbial balance between predominant groups was unchanged in CAs, as has been demonstrated previously[22]. These observations are similar to what Hester *et al*[50] noted in their investigation in that the *Firmicutes/Bacteroidetes* ratio is higher in AAs compared to CAs. There is also a relative abundance of *B. massiliensis* in AAs compared to CAs. CRC has also been shown to be associated *F. nucleatum* and pro-inflammatory bacteria, *Enterobacter* and *Clostridium XI*-species. *F. nucleatum* strains have been shown to promote carcinogenesis and invasion of host cells and potentiate tumorigenesis in mouse model of colon cancer[23,37]. CRC patients demonstrate a higher abundance of *F. nucleatum* and *Clostridium difficile,* a member of *Clostridium XI*[51,52]. Others have demonstrated that the relative increase in *Clostridium*

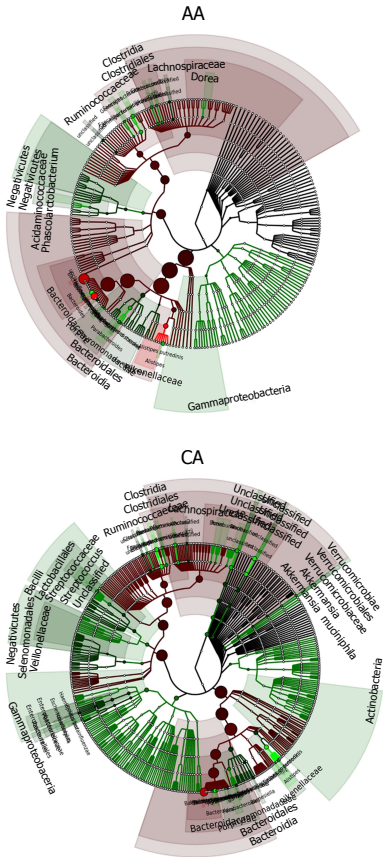

Figure 6 Phylogenetic tree showing the differences and abundance of taxa in African Americans and Caucasian Americans colonic effluents. The taxon size and color indicate the relative abundance of family. GraPhlAn software was used for taxonomic classification and circular taxonomic phylogenetic trees and Ribosomal Database Project classifier, Quantitative Insights into Microbial Ecology, R software/Tool were used for taxonomic data analysis.

cluster XI and *Enterobacteriaceae* are associated with intestinal dysbiosis[53].

Previous studies have suggested that a decrease in commensal microbiota in AAs may contribute to the tumorigenic microenvironment and that dysbiosis of gut microbiota may be partially responsible for promoting CRC and colitis-associated CRC[22,42,54]. In line with these observations, we found commensal bacteria *B. fragilis* to be slightly higher in CAs than AAs. *Bacteroides fragilis* is an immunomodulatory bacteria, which stimulates anti-inflammatory cytokine IL10 by Foxp3+ regulatory CD+ T (Treg) cells and suppresses mucosal inflammation[55,56]. In contrast, *Unclassified-Bacteria* and *Unclassified-Unclassified* micro-organisms were only present in CAs *A. municiphila,* a member of *Verrumicomicrobia,* is an intestinal symbiont and is known to induce an anti-inflammatory effect and enhance immune function[57]. Depletion of *A. muciniphila* is associated with a variety of diseases, including diabetes[58,59]. We found the relative abundance of *A. muciniphila* to be lower in AAs than their CA counterparts. Collectively, AAs have fewer bacterial populations that are known to suppress inflammation, improve mucus barrier function, and diminish permeability[60].

Human genetic variants can modulate the effects of the microbiome composition, and both are associated with many human complex diseases[61]. Microbiota changes with diet and stimulatory agents and can modulate disease development and progression[61,62]. Microbial dysbiosis in AAs may serve as a point for prevention and ultimately treatment of CRC. Identifying a microbial signature associated with CRC is complicated by many factors. This study was limited by focusing on a specific population with limited sample size and did not investigate dietary differences. However, there were significant differences between the colonic effluent microbiota of the AA and CA study groups - with less diversity of bacteria, greater abundance of pro-inflammatory bacteria, and reduced anti-inflammatory bacteria. Mechanisms for tumorigenesis may include bacteria that promote SBA transformation, as suggested by higher 7-α-dehydroxylase in the AA *vs* CA group. Further study is needed to evaluate the role of decreased diversity and structural imbalance in the colon microbial communities and the development of CRC.

ARTICLE HIGHLIGHTS

Research background

The incidence of colorectal cancer (CRC), the third most common malignancy, is not only higher among African Americans (AAs), but is also associated with higher mortality. In addition, AAs tend to be diagnosed with CRC at a younger age than Caucasian Americans (CAs) and exhibit worse prognoses than their CA counterparts. Despite this grim outlook, neither the extrinsic/intrinsic factor(s) nor the underlying molecular and/or biochemical mechanisms are fully understood. We hypothesize that imbalance in the gut microbiome between AAs and CAs results in alterations of metabolites, which changes symbiotic relationships and enhance gastrointestinal diseases, including CRC. A number of bacteria are known to promote carcinogenesis in the colon by altering gut microbial composition, which may play a major role in colorectal carcinogenesis.

Research motivation

CRC is the third leading malignancy world-wide, affecting both males and females equally. It represents one of the most common cancers in the United States and is estimated to be the second and third leading cause of cancer-related deaths in men and women, respectively, in the United States. Several studies have also demonstrated that AAs have the highest rate of CRC than any other racial group in the USA, and also AA men are even more likely to die from CRC than AA women. With these grim statistics, it is important to gain a better understanding of the underlying mechanism(s), particularly the role of gut microbiota, in regulating racial disparity in colorectal carcinogenesis.

Research objectives

The current investigation was aimed at studying microbial dysbiosis in the gut between AAs and CAs. The primary endpoint of this investigation was to determine whether the increased incidence of CRC in AAs could be attributed to alterations in gut microbiota. In this pilot study, we investigated the diversity and abundance of specific gut microbial communities in colonic effluents using 16S rRNA gene profiling in AAs and CAs and their possible role in the increased incidence of CRC in AAs.

Research methods

Male and female AA and CA patients, aged between 40 and 80 years, undergoing routine colonoscopy at the John D. Dingell VA Medical Center in Detroit were asked to participate. To determine the microbial diversity and the microbial richness in AAs and CAs, colonic effluent from each patient was used for DNA extraction and 16s RNA gene-based microbial community profiling which was performed and analyzed by LC Sciences (Houston, Texas, United

States). The composition of OTU and alpha diversity was measured by Venn diagram and Rarefaction measurement method. The relative abundance of phylum and classes was depicted by bar and pie chart. The phylogenic tree was plotted for AA and CA to determine the relative abundance of family in microbial community. Several inflammatory and probiotic bacterial marker candidates such as *Enterobacteria, Bifidobacteria, Lactobacillus, Fusobacterium* and/or *Clostridium genus* and species-specific bacteria were identified by real-time qPCR using specific primers designed on the basis of conserved and variable region in bacterial 16S rRNA genes according to our standard protocol. Statistical analysis was performed for each experiment accordingly.

Research results

The relative abundance of *Fusobacterium nucleatum,* which has been associated with the development and progression of CRC, was found to be significantly higher in AAs than CAs, indicating a greater risk for the development of CRC in AAs. *Clostridium IV,* a known mediator of anti-inflammatory effects, was found to be higher in CAs than AAs.

Research conclusion

The human colon harbors a complex microbial flora. Bacterial density in the human colon is among the highest found in nature, approaching 10^{12} bacteria/gm wet weight of feces. These bacteria are in a symbiotic relationship with the intestine, utilizing undigested nutrients as substrates and in return, produce various vitamins, amino acids, transform bile salts and assist in the maintenance of the intestinal barrier, and the appropriate immune response against pathogens. This homeostasis is altered in a state of dysbiosis, which is overgrowth of pathogenic bacteria that are normally inhibited by commensal bacteria. Our current investigation, for the first time, demonstrates microbial dysbiosis between AAs and CAs. This imbalance, we believe, is partially responsible for the racial disparity in CRC observed between AAs and CAs.

Research perspective

Although numerous studies have demonstrated that the incidence of CRC is higher in AAs than CAs, the reasons for this racial disparity are not fully understood. Data generated from this investigation reveal a role for the gut microbiome in racial disparity. The precise mechanisms by which changes in gut microbiota would lead to an increase CRC in AAs remain unexplored. However, it is tempting to speculate that this dysbiosis or overgrowth of certain bacteria in the gut of AAs resulting in alterations in microbial metabolites, specifically deoxycholic acid and lithocholic acid, which are known for their co-carcinogenic activity, could induce the process(es) of carcinogenesis in the colon of AAs. However, the levels of microbial metabolites, including bile acids in AAs and CAs with and without adenomas have not determined. Moreover, no information is available whether the observed dysbiosis in AAs is due to changes in diet and/or lifestyle. Undoubtedly, further investigations are needed to gain a better and fuller understanding of the intrinsic and extrinsic factors that are critically involved in regulating racial disparity in CRC.

REFERENCES

1 **Dimou A**, Syrigos KN, Saif MW. Disparities in colorectal cancer in African-Americans vs Whites: before and after diagnosis. *World J Gastroenterol* 2009; **15**: 3734-3743 [PMID: 19673013 DOI: 10.3748/wjg.15.3734]
2 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
3 **Lieberman DA**, Holub JL, Moravec MD, Eisen GM, Peters D, Morris CD. Prevalence of colon polyps detected by colonoscopy screening in asymptomatic black and white patients. *JAMA* 2008; **300**: 1417-1422 [PMID: 18812532 DOI: 10.1001/jama.300.12.1417]
4 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.20138]
5 **Wallace K**, Hill EG, Lewin DN, Williamson G, Oppenheimer S, Ford ME, Wargovich MJ, Berger FG, Bolick SW, Thomas MB, Alberg AJ. Racial disparities in advanced-stage colorectal cancer survival. *Cancer Causes Control* 2013; **24**: 463-471 [PMID: 23296454 DOI: 10.1007/s10552-012-0133-5]
6 **Wallace K**, Sterba KR, Gore E, Lewin DN, Ford ME, Thomas MB, Alberg AJ. Prognostic factors in relation to racial disparity in advanced colorectal cancer survival. *Clin Colorectal Cancer* 2013; **12**: 287-293 [PMID: 24188687 DOI: 10.1016/j.clcc.2013.08.001]
7 **Ashktorab H**, Kupfer SS, Brim H, Carethers JM. Racial Disparity in Gastrointestinal Cancer Risk. *Gastroenterology* 2017; **153**: 910-923 [PMID: 28807841 DOI: 10.1053/j.gastro.2017.08.018]
8 **Amri R**, Bordeianou LG, Berger DL. The conundrum of the young colon cancer patient. *Surgery* 2015; **158**: 1696-1703 [PMID: 26298030 DOI: 10.1016/j.surg.2015.07.018]
9 **Ashktorab H**, Vilmenay K, Brim H, Laiyemo AO, Kibreab A, Nouraie M. Colorectal Cancer in Young African Americans: Is It Time to Revisit Guidelines and Prevention? *Dig Dis Sci* 2016; **61**: 3026-3030 [PMID: 27278956 DOI: 10.1007/s10620-016-4207-1]
10 **Carethers JM**. Screening for colorectal cancer in African Americans: determinants and rationale for an earlier age to commence screening. *Dig Dis Sci* 2015; **60**: 711-721 [PMID: 25540085 DOI: 10.1007/s10620-014-3443-5]
11 **Laryea JA**, Siegel E, Klimberg S. Racial disparity in colorectal cancer: the role of equal treatment. *Dis Colon Rectum* 2014; **57**: 295-302 [PMID: 24509450 DOI: 10.1097/DCR.0000000000000056]
12 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767 [PMID: 2188735 DOI: 10.1016/0092-8674(90)90186-I]
13 **Vogelstein B**, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. *Science* 2013; **339**: 1546-1558 [PMID: 23539594 DOI: 10.1126/science.1235122]
14 **Todaro M**, Iovino F, Eterno V, Cammareri P, Gambara G, Espina V, Gulotta G, Dieli F, Giordano S, De Maria R, Stassi G. Tumorigenic and metastatic activity of human thyroid cancer stem cells. *Cancer Res* 2010; **70**: 8874-8885 [PMID: 20959469 DOI: 10.1158/0008-5472.CAN-10-1994]
15 **Farhana L**, Antaki F, Anees MR, Nangia-Makker P, Judd S, Hadden T, Levi E, Murshed F, Yu Y, Van Buren E, Ahmed K, Dyson G, Majumdar AP. Role of cancer stem cells in racial disparity in colorectal cancer. *Cancer Med* 2016; **5**: 1268-1278 [PMID: 26990997 DOI: 10.1002/cam4.690]
16 **Alexander DD**, Cushing CA, Lowe KA, Sceurman B, Roberts MA. Meta-analysis of animal fat or animal protein intake and colorectal cancer. *Am J Clin Nutr* 2009; **89**: 1402-1409 [PMID: 19261724 DOI: 10.3945/ajcn.2008.26838]
17 **Carethers JM**. Racial and ethnic factors in the genetic pathogenesis of colorectal cancer. *J Assoc Acad Minor Phys* 1999; **10**: 59-67 [PMID: 10826011]
18 **Huxley RR**, Ansary-Moghaddam A, Clifton P, Czernichow S, Parr CL, Woodward M. The impact of dietary and lifestyle risk factors on risk of colorectal cancer: a quantitative overview of the epidemiological evidence. *Int J Cancer* 2009; **125**: 171-180 [PMID: 19350627 DOI: 10.1002/ijc.24343]
19 **Sandler RS**. Aspirin and other nonsteroidal anti-inflammatory agents in the prevention of colorectal cancer. *Important Adv Oncol* 1996: 123-137 [PMID: 8791132]
20 **Dethlefsen L**, Eckburg PB, Bik EM, Relman DA. Assembly of the human intestinal microbiota. *Trends Ecol Evol* 2006; **21**: 517-523 [PMID: 16820245 DOI: 10.1016/j.tree.2006.06.013]
21 **Abreu MT**, Peek RM Jr. Gastrointestinal malignancy and the microbiome. *Gastroenterology* 2014; **146**: 1534-1546.e3 [PMID: 24406471 DOI: 10.1053/j.gastro.2014.01.001]
22 **Gagnière J**, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E, Bringer MA, Pezet D, Bonnet M. Gut microbiota imbalance and colorectal cancer. *World J Gastroenterol* 2016; **22**: 501-518 [PMID: 26811603 DOI: 10.3748/wjg.v22.i2.501]
23 **Kostic AD**, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, Clancy TE, Chung DC, Lochhead P, Hold GL, El-Omar EM, Brenner D, Fuchs CS, Meyerson M, Garrett WS. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 2013; **14**: 207-215 [PMID: 23954159 DOI: 10.1016/j.chom.2013.07.007]
24 **Tözün N**, Vardareli E. Gut Microbiome and Gastrointestinal Cancer: Les liaisons Dangereuses. *J Clin Gastroenterol* 2016; **2015**: S191-S196 [PMID: 27741173 DOI: 10.1097/MCG.0000000000000714]
25 **Boutagy NE**, Neilson AP, Osterberg KL, Smithson AT, Englund TR,

Davy BM, Hulver MW, Davy KP. Short-term high-fat diet increases postprandial trimethylamine-N-oxide in humans. *Nutr Res* 2015; **35**: 858-864 [PMID: 26265295 DOI: 10.1016/j.nutres.2015.07.002]

26 **Fleming SE**, Fitch MD, Chansler MW. High-fiber diets: influence on characteristics of cecal digesta including short-chain fatty acid concentrations and pH. *Am J Clin Nutr* 1989; **50**: 93-99 [PMID: 2546417 DOI: 10.1093/ajcn/50.1.93]

27 **Northfield TC**, McColl I. Postprandial concentrations of free and conjugated bile acids down the length of the normal human small intestine. *Gut* 1973; **14**: 513-518 [PMID: 4729918 DOI: 10.1136/gut.14.7.513]

28 **Zitvogel L**, Pietrocola F, Kroemer G. Nutrition, inflammation and cancer. *Nat Immunol* 2017; **18**: 843-850 [PMID: 28722707 DOI: 10.1038/ni.3754]

29 **Ajouz H**, Mukherji D, Shamseddine A. Secondary bile acids: an underrecognized cause of colon cancer. *World J Surg Oncol* 2014; **12**: 164 [PMID: 24884764 DOI: 10.1186/1477-7819-12-164]

30 **Narisawa T**, Magadia NE, Weisburger JH, Wynder EL. Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation of N-methyl-N'-nitro-N-nitrosoguanidine in rats. *J Natl Cancer Inst* 1974; **53**: 1093-1097 [PMID: 4427390 DOI: 10.1093/jnci/53.4.1093]

31 **Ou J**, DeLany JP, Zhang M, Sharma S, O'Keefe SJ. Association between low colonic short-chain fatty acids and high bile acids in high colon cancer risk populations. *Nutr Cancer* 2012; **64**: 34-40 [PMID: 22136517 DOI: 10.1080/01635581.2012.630164]

32 **Payne CM**, Crowley-Skillicorn C, Bernstein C, Holubec H, Moyer MP, Bernstein H. Hydrophobic bile acid-induced micronuclei formation, mitotic perturbations, and decreases in spindle checkpoint proteins: relevance to genomic instability in colon carcinogenesis. *Nutr Cancer* 2010; **62**: 825-840 [PMID: 20661832 DOI: 10.1080/01635581003695756]

33 **Reddy BS**, Martin CW, Wynder EL. Fecal bile acids and cholesterol metabolites of patients with ulcerative colitis, a high-risk group for development of colon cancer. *Cancer Res* 1977; **37**: 1697-1701 [PMID: 322859]

34 **Cao H**, Xu M, Dong W, Deng B, Wang S, Zhang Y, Wang S, Luo S, Wang W, Qi Y, Gao J, Cao X, Yan F, Wang B. Secondary bile acid-induced dysbiosis promotes intestinal carcinogenesis. *Int J Cancer* 2017; **140**: 2545-2556 [PMID: 28187526 DOI: 10.1002/ijc.30643]

35 **Wells JE**, Williams KB, Whitehead TR, Heuman DM, Hylemon PB. Development and application of a polymerase chain reaction assay for the detection and enumeration of bile acid 7alpha-dehydroxylating bacteria in human feces. *Clin Chim Acta* 2003; **331**: 127-134 [PMID: 12691873 DOI: 10.1016/S0009-8981(03)00115-3]

36 **Mira-Pascual L**, Cabrera-Rubio R, Ocon S, Costales P, Parra A, Suarez A, Moris F, Rodrigo L, Mira A, Collado MC. Microbial mucosal colonic shifts associated with the development of colorectal cancer reveal the presence of different bacterial and archaeal biomarkers. *J Gastroenterol* 2015; **50**: 167-179 [PMID: 24811328 DOI: 10.1007/s00535-014-0963-x]

37 **Rubinstein MR**, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. *Cell Host Microbe* 2013; **14**: 195-206 [PMID: 23954158 DOI: 10.1016/j.chom.2013.07.012]

38 **Arpaia N**, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffer PJ, Rudensky AY. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013; **504**: 451-455 [PMID: 24226773 DOI: 10.1038/nature12726]

39 **Furusawa Y**, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013; **504**: 446-450 [PMID: 24226770 DOI: 10.1038/nature12721]

40 **Smith PM**, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, Glickman JN, Garrett WS. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 2013; **341**: 569-573 [PMID: 23828891 DOI: 10.1126/science.1241165]

41 **Ridlon JM**, Kang DJ, Hylemon PB, Bajaj JS. Bile acids and the gut microbiome. *Curr Opin Gastroenterol* 2014; **30**: 332-338 [PMID: 24625896 DOI: 10.1097/MOG.0000000000000057]

42 **Nakatsu G**, Li X, Zhou H, Sheng J, Wong SH, Wu WK, Ng SC, Tsoi H, Dong Y, Zhang N, He Y, Kang Q, Cao L, Wang K, Zhang J, Liang Q, Yu J, Sung JJ. Gut mucosal microbiome across stages of colorectal carcinogenesis. *Nat Commun* 2015; **6**: 8727 [PMID: 26515465 DOI: 10.1038/ncomms9727]

43 **Sanapareddy N**, Legge RM, Jovov B, McCoy A, Burcal L, Araujo-Perez F, Randall TA, Galanko J, Benson A, Sandler RS, Rawls JF, Abdo Z, Fodor AA, Keku TO. Increased rectal microbial richness is associated with the presence of colorectal adenomas in humans. *ISME J* 2012; **6**: 1858-1868 [PMID: 22622349 DOI: 10.1038/ismej.2012.43]

44 **Ahn J**, Sinha R, Pei Z, Dominianni C, Wu J, Shi J, Goedert JJ, Hayes RB, Yang L. Human gut microbiome and risk for colorectal cancer. *J Natl Cancer Inst* 2013; **105**: 1907-1911 [PMID: 24316595 DOI: 10.1093/jnci/djt300]

45 **Peters BA**, Dominianni C, Shapiro JA, Church TR, Wu J, Miller G, Yuen E, Freiman H, Lustbader I, Salik J, Friedlander C, Hayes RB, Ahn J. The gut microbiota in conventional and serrated precursors of colorectal cancer. *Microbiome* 2016; **4**: 69 [PMID: 28038683 DOI: 10.1186/s40168-016-0218-6]

46 **Hale VL**, Chen J, Johnson S, Harrington SC, Yab TC, Smyrk TC, Nelson H, Boardman LA, Druliner BR, Levin TR, Rex DK, Ahnen DJ, Lance P, Ahlquist DA, Chia N. Shifts in the Fecal Microbiota Associated with Adenomatous Polyps. *Cancer Epidemiol Biomarkers Prev* 2017; **26**: 85-94 [PMID: 27672054 DOI: 10.1158/1055-9965.EPI-16-0337]

47 **Shen XJ**, Rawls JF, Randall T, Burcal L, Mpande CN, Jenkins N, Jovov B, Abdo Z, Sandler RS, Keku TO. Molecular characterization of mucosal adherent bacteria and associations with colorectal adenomas. *Gut Microbes* 2010; **1**: 138-147 [PMID: 20740058 DOI: 10.4161/gmic.1.3.12360]

48 **Allen-Vercoe E**, Jobin C. Fusobacterium and Enterobacteriaceae: important players for CRC? *Immunol Lett* 2014; **162**: 54-61 [PMID: 24972311 DOI: 10.1016/j.imlet.2014.05.014]

49 **Baxter NT**, Zackular JP, Chen GY, Schloss PD. Structure of the gut microbiome following colonization with human feces determines colonic tumor burden. *Microbiome* 2014; **2**: 20 [PMID: 24967088 DOI: 10.1186/2049-2618-2-20]

50 **Hester CM**, Jala VR, Langille MG, Umar S, Greiner KA, Haribabu B. Fecal microbes, short chain fatty acids, and colorectal cancer across racial/ethnic groups. *World J Gastroenterol* 2015; **21**: 2759-2769 [PMID: 25759547 DOI: 10.3748/wjg.v21.i9.2759]

51 **Franks AH**, Harmsen HJ, Raangs GC, Jansen GJ, Schut F, Welling GW. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl Environ Microbiol* 1998; **64**: 3336-3345 [PMID: 9726880]

52 **Fukugaiti MH**, Ignacio A, Fernandes MR, Ribeiro Júnior U, Nakano V, Avila-Campos MJ. High occurrence of Fusobacterium nucleatum and Clostridium difficile in the intestinal microbiota of colorectal carcinoma patients. *Braz J Microbiol* 2015; **46**: 1135-1140 [PMID: 26691472 DOI: 10.1590/S1517-838246420140665]

53 **Marteau P**, Chaput U. Bacteria as trigger for chronic gastrointestinal disorders. *Dig Dis* 2011; **29**: 166-171 [PMID: 21734380 DOI: 10.1159/000323879]

54 **Sobhani I**, Amiot A, Le Baleur Y, Levy M, Auriault ML, Van Nhieu JT, Delchier JC. Microbial dysbiosis and colon carcinogenesis: could colon cancer be considered a bacteria-related disease? *Therap Adv Gastroenterol* 2013; **6**: 215-229 [PMID: 23634186 DOI: 10.1177/1756283X12473674]

55 **Donaldson GP**, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol* 2016; **14**: 20-32 [PMID: 26499895 DOI: 10.1038/nrmicro3552]

56 **Zitvogel L**, Daillère R, Roberti MP, Routy B, Kroemer G. Anticancer effects of the microbiome and its products. *Nat Rev Microbiol* 2017; **15**: 465-478 [PMID: 28529325 DOI: 10.1038/nrmicro.2017.44]

57 **de Vos WM**. Microbe Profile: Akkermansia muciniphila: a conserved intestinal symbiont that acts as the gatekeeper of our mucosa. *Microbiology* 2017; **163**: 646-648 [PMID: 28530168 DOI: 10.1099/mic.0.000444]

58 **Belzer C**, de Vos WM. Microbes inside--from diversity to function: the case of Akkermansia. *ISME J* 2012; **6**: 1449-1458 [PMID: 22437156 DOI: 10.1038/ismej.2012.6]

59 **Plovier H**, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, Chilloux J, Ottman N, Duparc T, Lichtenstein L, Myridakis A, Delzenne NM, Klievink J, Bhattacharjee A, van der Ark KC, Aalvink S, Martinez LO, Dumas ME, Maiter D, Loumaye A, Hermans MP, Thissen JP, Belzer C, de Vos WM, Cani PD. A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med* 2017; **23**: 107-113 [PMID: 27892954 DOI: 10.1038/nm.4236]

60 **Plotnikoff GA**. Three measurable and modifiable enteric microbial biotransformations relevant to cancer prevention and treatment. *Glob Adv Health Med* 2014; **3**: 33-43 [PMID: 24891992 DOI: 10.7453/gahmj.2014.021]

61 **Luca F**, Kupfer SS, Knights D, Khoruts A, Blekhman R. Functional Genomics of Host-Microbiome Interactions in Humans. *Trends Genet* 2018; **34**: 30-40 [PMID: 29107345 DOI: 10.1016/j.tig.2017.10.001]

62 **Ou J**, Carbonero F, Zoetendal EG, DeLany JP, Wang M, Newton K, Gaskins HR, O'Keefe SJ. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *Am J Clin Nutr* 2013; **98**: 111-120 [PMID: 23719549 DOI: 10.3945/ajcn.112.056689]

63 **Yoshimoto S**, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, Iwakura Y, Oshima K, Morita H, Hattori M, Honda K, Ishikawa Y, Hara E, Ohtani N. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013; **499**: 97-101 [PMID: 23803760 DOI: 10.1038/nature12347]

64 **Matsuki T**, Watanabe K, Fujimoto J, Miyamoto Y, Takada T, Matsumoto K, Oyaizu H, Tanaka R. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. *Appl Environ Microbiol* 2002; **68**: 5445-5451 [PMID: 12406736 DOI: 10.1128/AEM.68.11.5445-5451.2002]

65 **Matsuki T**, Watanabe K, Fujimoto J, Takada T, Tanaka R. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. *Appl Environ Microbiol* 2004; **70**: 7220-7228 [PMID: 15574920 DOI: 10.1128/AEM.70.12.7220-7228.2004]

66 **Collado MC**, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am J Clin Nutr* 2008; **88**: 894-899 [PMID: 18842773 DOI: 10.1093/ajcn/88.4.894]

67 **Kostic AD**, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, Ojesina AI, Jung J, Bass AJ, Tabernero J, Baselga J, Liu C, Shivdasani RA, Ogino S, Birren BW, Huttenhower C, Garrett WS, Meyerson M. Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. *Genome Res* 2012; **22**: 292-298 [PMID: 22009990 DOI: 10.1101/gr.126573.111]

68 **Castellarin M**, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, Barnes R, Watson P, Allen-Vercoe E, Moore RA, Holt RA. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. *Genome Res* 2012; **22**: 299-306 [PMID: 22009989 DOI: 10.1101/gr.126516.111]

P- Reviewer: Huerta-Franco M, Mann O, Maric I, McMillin MA
S- Editor: Cui LJ **L- Editor**: Filipodia **E- Editor**: Yin SY

Published by **Baishideng Publishing Group Inc**
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Telephone: +1-925-223-8242
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ISSN 2150-5330 (online)

World Journal of *Gastrointestinal Pathophysiology*

World J Gastrointest Pathophysiol 2018 October 25; 9(3): 59-72

Published by Baishideng Publishing Group Inc

WJGP

World Journal of Gastrointestinal Pathophysiology

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World Journal of Gastrointestinal Pathophysiology
Volume 9 Number 3 October 25, 2018

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INDEXING/ABSTRACTING

World Journal of Gastrointestinal Pathophysiology is now abstracted and indexed in Emerging Sources Citation Index (Web of Science), PubMed, PubMed Central, China National Knowledge Infrastructure (CNKI), and Superstar Journals Database.

NAME OF JOURNAL
World Journal of Gastrointestinal Pathophysiology

ISSN
ISSN 2150-5330 (online)

LAUNCH DATE
April 15, 2010

Frequency
Quarterly

EDITORIAL OFFICE
Jin-Lei Wang, Director
World Journal of Gastrointestinal Pathophysiology
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
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PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive,
Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
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PUBLICATION DATE
October 25, 2018

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Gastrointestinal Pathophysiology

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World J Gastrointest Pathophysiol 2018 October 25; 9(3): 59-62

DOI: 10.4291/wjgp.v9.i3.59

ISSN 2150-5330 (online)

EDITORIAL

Calcium and vitamin D in the serrated neoplastic pathway: Friends or foes?

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Author contributions: García-Morales N drafted the initial manuscript; Satorres C performed the literature research and reviewed the manuscript draft; Bustamante-Balén M contributed to conception and design of the project and revised the article critically for important intellectual content.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Manuscript source: Invited Manuscript

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Received: July 24, 2018
Peer-review started: July 24, 2018
First decision: August 8, 2018
Revised: September 11, 2018
Accepted: October 9, 2018
Article in press: October 9, 2018
Published online: October 25, 2018

Abstract

Sessile serrated adenoma/polyps (known as SSA/Ps) may play an important role in the development of interval colorectal cancer (CRC). These lesions are more difficult to detect with conventional endoscopy and they may quickly turn into CRC, especially when dysplasia has developed. Therefore, primary or secondary chemoprevention may be an appealing strategy at a population level. Calcium and vitamin D have been shown in epidemiological studies to reduce the risk of CRC and conventional adenomas, but the evidence regarding their effect on SSA/Ps is controversial. In this editorial we comment on the results of a recent randomized controlled trial investigating the effect of calcium and vitamin D on the development of serrated lesions, summarizing the possible antineoplastic mechanisms of calcium and vitamin D, and discussing the differences found with previous observational reports.

Key words: Serrated polyps; Sessile serrated polyp; Vitamin D; Calcium; Colorectal cancer

Core tip: Calcium and vitamin D have been shown in epidemiological studies to reduce the risk of colorectal cancer and adenomas, but the evidence regarding their effect on sessile serrated adenomas/polyps (SSA/P) is controversial - some studies showing no effect and others showing some degree of risk reduction. Recently, a randomized controlled trial with calcium and

vitamin D supplements was published, concluding that the relative risk of developing a SSA/P was increased in patients taking calcium and vitamin D/calcium. In this editorial we try to place these surprising results into context, describing the limitations of this and previous studies on this topic.

García-Morales N, Satorres C, Bustamante-Balén M. Calcium and vitamin D in the serrated neoplastic pathway: Friends or foes? *World J Gastrointest Pathophysiol* 2018; 9(3): 59-62 Available from: URL: http://www.wjgnet.com/2150-5330/full/v9/i3/59.htm DOI: http://dx.doi.org/10.4291/wjgp.v9.i3.59

INTRODUCTION

Serrated polyps (SPs), and particularly sessile serrated adenoma/polyps (SSA/Ps), are precursor lesions of colorectal cancer (CRC). Some authors have affirmed that the so-called "serrated neoplastic pathway" is the route through which 10% to 30% of CRCs develop[1]. SSA/Ps are more difficult to detect with conventional endoscopy[2] and they may quickly turn into CRC, especially when dysplasia has developed[3]. Therefore, SSA/Ps may play an important role in the development of interval cancer. In this situation, primary or secondary chemoprevention may be an appealing strategy at a population level.

Calcium and vitamin D have been shown to exert their anticancer properties by stimulating differentiation, reducing proliferation and inducing apoptosis. The majority of epidemiologic studies support a reduction of the risk of CRC and adenomas by almost 30% when comparing high to low intake of both calcium and vitamin D. For instance, a dose-response meta-analysis of observational studies found that an intake of supplemental calcium could reduce the risk of CRC at a rate of 9% for each 300 mg/d increase[4]. Calcium supplementation has also been shown in a randomized trial to reduce the recurrence of colorectal adenomas[5].

Based on this idea, interest has been focused in recent years on the effect of calcium and vitamin D on SPs' development. Several large pooled studies and meta-analyses have been published on the topic, with disparate results. Some have shown no effect of calcium supplementation on SPs[6], others have shown a nonsignificant reduction in SP risk in individuals consuming the highest levels of calcium but no effect of vitamin D[7], and in other reports no effect of calcium was shown but vitamin D intake was found to be inversely associated with SPs, especially for polyps in the distal colon[8].

Despite the inclusion of hundreds of thousands of patients, these studies have some limitations that make it difficult to draw firm conclusions. Many studies were published years ago, prior to the classification of SPs into different subtypes. Current knowledge includes the different subtypes of SPs [hyperplastic polyp (HP), SSA/P, traditional serrated adenoma] being biologically different, posing different risks of developing a CRC, and possibly having different behaviors regarding dietary factors. Some of the included studies are based on sigmoidoscopies and, as we know, SSA/Ps are generally located in proximal segments of the colon[9]. Assessing possible risk factors in observational cohort studies may be difficult when there is a low prevalence of the disease - SSA/Ps in this case - while the prevalence of SSA/Ps in several studies is at most 8%[10]. In order to detect weak associations and describe possible long-term effects, follow-up should be long enough; however, most prospective studies have a relatively short follow-up, between 1 to 6 year[5,7]. Finally, risk factors often overlap in dietary epidemiological studies and, in regards to the specific case of calcium and vitamin D; many studies do not assess them separately.

Recently, a colonoscopy-based case-control study analyzing data from more than 7000 patients and controls was published[11]. In this study, an expert pathologist classified all serrated lesions according to subtype. Among other lifestyle and dietary factors, calcium intake was associated with a reduced risk of HP and adenomas but not with a statistically significant reduction in the risk of SSA/Ps. Vitamin D intake was not considered. Although designed following up-to-date knowledge, this study again has some limitations that do not allow the definite ruling out of a possible influence of calcium intake on SP development. Based on surveys, recall bias cannot be excluded. Moreover, only those individuals who answered some surveys were included, representing only 51% of the initial candidates. Sample size could also be seen as an issue, since patients with SSA/Ps accounted for around only 7% of the entire case group. Finally, in this study only dietary calcium, and not supplements, was evaluated.

All together, these observational and case-control studies are a very useful tool for detecting possible associations and formulating hypothesis, but casualty has to be confirmed in clinical trials. This is the reason why the results of a well-designed randomized controlled trial have been eagerly awaited.

A randomized, multicenter, double-blind, placebo-controlled chemoprevention trial with calcium and vitamin D supplements was recently published. Crockett *et al*[11] analyzed the risk of SPs among participants in the Vitamin D/Calcium Polyp Prevention Study. Participants with at least one adenoma in a baseline colonoscopy were included and distributed in four treatment arms (calcium, vitamin D, both or placebo). Individuals were treated for 3-5 year (treatment phase, *n* = 2058 patients), until the next surveillance colonoscopy, enabling a complete follow-up of at least 3 more year (observation phase, *n* = 1108 patients). A total of 1111 SPs (955 of HP and 132 of SSA/P) and 607 SPs (498 of HP and 79 SSA/Ps) were detected at the end of the treatment phase and the observation phase, respectively. There was no difference in the risk of developing a SP in patients taking vitamin D, calcium

or vitamin D plus calcium during the treatment phase. However, during the observation phase, relative risk of developing a SSA/P had increased in patients taking calcium and vitamin D plus calcium [crude relative risk: 2.72 (1.47-5.03) and 4.09 (1.6-10.5), respectively]. This risk was further increased in women and smokers.

This study shows surprising results, as they go against previous findings, and they also seem to contradict current knowledge regarding the role of vitamin D in cancer prevention. This latter aspect is particularly intriguing. Why has vitamin D been related to an anti-neoplastic activity? Skin-produced vitamin D_3 goes through two-cytochrome P450-mediated hydroxylation steps, first in the liver and then in the kidney, to yield calcitriol. Calcitriol - besides its critical role in regulating mineral homeostasis - through its binding to the nuclear vitamin D receptor (VDR), modulates the expression of many genes, thereby regulating multiple signaling pathways affecting inflammation, cell differentiation and proliferation, apoptotic mechanisms, invasion and metastasis[12]. The CYP27B1 enzyme, responsible for the second step of hydrolization in the kidney, has been shown to be present in several extra-kidney tissues and in cancer cells as well. Modulation of signaling pathways at this level could be responsible for vitamin D anticancer properties.

In the case of CRC, these properties are mainly driven by the modulation of the Wnt-β-catenin pathway[12]. Among other mechanisms, VDR binds to β-catenin, inhibiting its nuclear translocation. Wnt activation has been demonstrated in 93% of CRC[13]. Therefore, its inhibition could be keying in the anticancer properties of vitamin D. However, the role of this VDR-mediated mechanism in the modulation of SPs development is not so clear. The Wnt pathway has been related to the transition to dysplasia in SPs, according to β-catenin immunostaining being more prevalent in dysplastic lesions[14]. Therefore, the Wnt pathway does not seem to be essential in the earlier steps of the serrated pathway, and its inhibition could not therefore affect the overall incidence of SPs.

And, what about the main molecular mechanisms involved in the serrated pathway? These mechanisms are aberrant promoter hypermethylation of CpG islands (CIMP phenotype), microsatellite instability (MSI), and alteration of the mitogen-activated protein kinase pathway (*BRAF* and *KRAS* mutations). Few studies have assessed the relationship between vitamin D and calcium and these molecular alterations, but a consistent effect of these nutrients has not been shown. For instance, there is a study showing how VDR over-expression was significantly associated with *KRAS* mutation but not with *BRAF* mutation, CIMP or MSI[15]. In another study, calcium intake was not associated with CIMP status[16]. In a case-control study, neither calcium nor vitamin D was related to the MSI status[17]. Evidence for the effect of calcium in other putative molecular alterations of SPs is even weaker.

It is hard to explain the difference between the results of Crockett *et al*[11] and those of previous reports. Unlike most observational studies, the effect of vitamin D and calcium could be separately assessed. Moreover, supplements of both nutrients were given in this therapeutic trial, and their effect could be different from that of daily intake. Another reason may be that this trial assesses the effect of calcium and vitamin D on incidental polyps, while observational studies assess the effect on prevalent ones. Inherent limitations of this trial should be also taken into account. The study is a secondary analysis of the Vitamin D/Calcium Polyp Prevention Study, initially designed to evaluate the risk of adenomas. The final sample size of SSA/Ps was small and many of the subgroup analysis may be under-powered, as the authors acknowledge. Another limitation is that only 53.8% of patients in the treatment phase provided enough information to be evaluated in the observation phase.

CONCLUSION

In conclusion, this study raises more questions than it provides answers. Just as the use of calcium and vitamin D as chemopreventive agents could not be recommended on the basis of the results of observational studies, its avoidance in certain groups to decrease the incidence of SSA/Ps should not be recommended either. At the moment, we cannot decide if calcium and vitamin D are friends or foes, but this study reminds us that, albeit necessary, observational studies do not give us the same level of evidence as a well-designed randomized controlled trial does. Calcium and vitamin D supplements are widely used at a population level. Lessons learned from this trial should prompt the design of more powerful, multicenter, randomized trials to finally clarify whether their use should be recommended or discouraged.

REFERENCES

1 **Snover DC**. Update on the serrated pathway to colorectal carcinoma. *Hum Pathol* 2011; **42**: 1-10 [PMID: 20869746 DOI: 10.1016/j.humpath.2010.06.002]

2 **East JE**, Vieth M, Rex DK. Serrated lesions in colorectal cancer screening: detection, resection, pathology and surveillance. *Gut* 2015; **64**: 991-1000 [PMID: 25748647 DOI: 10.1136/gutjnl-2014-309041]

3 **Oono Y**, Fu K, Nakamura H, Iriguchi Y, Yamamura A, Tomino Y, Oda J, Mizutani M, Takayanagi S, Kishi D, Shinohara T, Yamada K, Matumoto J, Imamura K. Progression of a sessile serrated adenoma to an early invasive cancer within 8 months. *Dig Dis Sci* 2009; **54**: 906-909 [PMID: 18688718 DOI: 10.1007/s10620-008-0407-7]

4 **Keum N**, Aune D, Greenwood DC, Ju W, Giovannucci EL. Calcium intake and colorectal cancer risk: dose-response meta-analysis of prospective observational studies. *Int J Cancer* 2014; **135**: 1940-1948 [PMID: 24623471 DOI: 10.1002/ijc.28840]

5 **Baron JA**, Beach M, Mandel JS, van Stolk RU, Haile RW, Sandler RS, Rothstein R, Summers RW, Snover DC, Beck GJ, Bond JH, Greenberg ER. Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. *N Engl J Med* 1999; **340**: 101-107 [PMID: 9887161 DOI: 10.1056/

NEJM199901143400204]
6 **Wallace K**, Grau MV, Ahnen D, Snover DC, Robertson DJ, Mahnke D, Gui J, Barry EL, Summers RW, McKeown-Eyssen G, Haile RW, Baron JA. The association of lifestyle and dietary factors with the risk for serrated polyps of the colorectum. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 2310-2317 [PMID: 19661090 DOI: 10.1158/1055-9965.EPI-09-0211]
7 **Bailie L**, Loughrey MB, Coleman HG. Lifestyle Risk Factors for Serrated Colorectal Polyps: A Systematic Review and Meta-analysis. *Gastroenterology* 2017; **152**: 92-104 [PMID: 27639804 DOI: 10.1053/j.gastro.2016.09.003]
8 **He X**, Wu K, Ogino S, Giovannucci EL, Chan AT, Song M. Association Between Risk Factors for Colorectal Cancer and Risk of Serrated Polyps and Conventional Adenomas. *Gastroenterology* 2018; **155**: 355-373.e18 [PMID: 29702117 DOI: 10.1053/j.gastro.2018.04.019]
9 **Hetzel JT**, Huang CS, Coukos JA, Omstead K, Cerda SR, Yang S, O'Brien MJ, Farraye FA. Variation in the detection of serrated polyps in an average risk colorectal cancer screening cohort. *Am J Gastroenterol* 2010; **105**: 2656-2664 [PMID: 20717107 DOI: 10.1038/ajg.2010.315]
10 **Abdeljawad K**, Vemulapalli KC, Kahi CJ, Cummings OW, Snover DC, Rex DK. Sessile serrated polyp prevalence determined by a colonoscopist with a high lesion detection rate and an experienced pathologist. *Gastrointest Endosc* 2015; **81**: 517-524 [PMID: 24998465 DOI: 10.1016/j.gie.2014.04.064]
11 **Crockett SD**, Barry EL, Mott LA, Ahnen DJ, Robertson DJ, Anderson JC, Wallace K, Burke CA, Bresalier RS, Figueiredo JC, Snover DC, Baron JA. Calcium and vitamin D supplementation and increased risk of serrated polyps: results from a randomised clinical trial. *Gut* 2018; [PMID: 29496722 DOI: 10.1136/gutjnl-2017-315242]
12 **Feldman D**, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. *Nat Rev Cancer* 2014; **14**: 342-357 [PMID: 24705652 DOI: 10.1038/nrc3691]
13 **Cancer Genome Atlas Network.** Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 330-337 [PMID: 22810696 DOI: 10.1038/nature11252]
14 **Yachida S**, Mudali S, Martin SA, Montgomery EA, Iacobuzio-Donahue CA. Beta-catenin nuclear labeling is a common feature of sessile serrated adenomas and correlates with early neoplastic progression after BRAF activation. *Am J Surg Pathol* 2009; **33**: 1823-1832 [PMID: 19745699 DOI: 10.1097/PAS.0b013e3181b6da19]
15 **Kure S**, Nosho K, Baba Y, Irahara N, Shima K, Ng K, Meyerhardt JA, Giovannucci EL, Fuchs CS, Ogino S. Vitamin D receptor expression is associated with PIK3CA and KRAS mutations in colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 2765-2772 [PMID: 19789368 DOI: 10.1158/1055-9965.EPI-09-0490]
16 **Weisenberger DJ**, Levine AJ, Long TI, Buchanan DD, Walters R, Clendenning M, Rosty C, Joshi AD, Stern MC, LeMarchand L, Lindor NM, Daftary D, Gallinger S, Selander T, Bapat B, Newcomb PA, Campbell PT, Casey G, Ahnen DJ, Baron JA, Haile RW, Hopper JL, Young JP, Laird PW, Siegmund KD; Colon Cancer Family Registry. Association of the colorectal CpG island methylator phenotype with molecular features, risk factors, and family history. *Cancer Epidemiol Biomarkers Prev* 2015; **24**: 512-519 [PMID: 25587051 DOI: 10.1158/1055-9965.EPI-14-1161]
17 **Slattery ML**, Anderson K, Curtin K, Ma KN, Schaffer D, Samowitz W. Dietary intake and microsatellite instability in colon tumors. *Int J Cancer* 2001; **93**: 601-607 [PMID: 11477566]

P- Reviewer: Mann O, Nishida T **S- Editor**: Dou Y **L- Editor**: A
E- Editor: Huang Y

World Journal of Gastrointestinal Pathophysiology

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World J Gastrointest Pathophysiol 2018 October 25; 9(3): 63-72

DOI: 10.4291/wjgp.v9.i3.63

ISSN 2150-5330 (online)

MINIREVIEWS

Gastro-oesophageal reflux disease and eosinophilic oesophagitis: What is the relationship?

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Author contributions: Wong S contributed to drafting of manuscript; Ruszkiewicz A, Holloway RH and Nguyen NQ contributed to critical revisions of manuscript.

Conflict-of-interest statement: There is no conflict of interest to disclose from all authors.

Manuscript source: Invited manuscript

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Received: August 2, 2018
Peer-review started: August 3, 2018
First decision: August 24, 2018
Revised: September 17, 2018
Accepted: October 11, 2018
Article in press: October 11, 2018
Published online: October 25, 2018

Abstract

Eosinophilic oesophagitis (EoE) and gastro-oesophageal reflux disease (GORD) are the most common causes of chronic oesophagitis and dysphagia associated with oesophageal mucosal eosinophilia. Distinguishing between the two is imperative but challenging due to overlapping clinical and histological features. A diagnosis of EoE requires clinical, histological and endoscopic correlation whereas a diagnosis of GORD is mainly clinical without the need for other investigations. Both entities may exhibit oesophageal eosinophilia at a similar level making a histological distinction between them difficult. Although the term proton-pump inhibitor responsive oesophageal eosinophilia has recently been retracted from the guidelines, a relationship between EoE and GORD still exists. This relationship is complex as they may coexist, either interacting bidirectionally or are unrelated. This review aims to outline the differences and potential relationship between the two conditions, with specific focus on histology, immunology, pathogenesis and treatment.

Key words: Relationship; Pathogenesis; Eosinophilic oesophagitis; Histological features; Gastro-oesophageal reflux disease

Core tip: The relationship between gastro-oesophageal reflux disease and eosinophilic oesophagitis is complex as they may coexist, either interacting bidirectionally or are

unrelated. This review aims to outline the differences and potential relationship between the two conditions, with specific focus on histology, immunology, pathogenesis and treatment.

Wong S, Ruszkiewicz A, Holloway RH, Nguyen NQ. Gastro-oesophageal reflux disease and eosinophilic oesophagitis: What is the relationship? *World J Gastrointest Pathophysiol* 2018; 9(3): 63-72 Available from: URL: http://www.wjgnet.com/2150-5330/full/v9/i3/63.htm DOI: http://dx.doi.org/10.4291/wjgp.v9.i3.63

INTRODUCTION

Eosinophilic oesophagitis (EoE) is a clinicopathological condition characterised by an antigen-driven immunologic process that manifests clinically with symptoms of oesophageal dysfunction and histologically by eosinophilic inflammation[1]. The first case report of oesophageal eosinophilia can be traced back as far as 1962 by Schreiber[2], followed by the first published case series of EoE as a distinct clinicopathological condition in 1993 by Attwood *et al*[3] In 2007, the first consensus recommendation by an international expert panel for the diagnosis and treatment of EoE was published[4]. This consensus was recently updated in 2017[5].

The recognition of EoE has increased so swiftly that it is now thought to be the most frequent eosinophilic gastrointestinal disorder as well as the second most common cause of chronic oesophagitis and dysphagia after gastro-oesophageal reflux disease (GORD)[6]. Although it is still an uncommon disease, the prevalence has been increasing over the past few years with an estimated prevalence in the general population of 13-49 cases/100000 persons[5,7]. This is also in keeping with an increasing incidence of EoE estimated at 1-20 cases/100000 persons[5,7]. Various hypotheses have been considered for this phenomenon particularly that of an increase in the recognition of the disease and an increase in volume of endoscopies performed[8-10]. However, two population-based studies have shown that the incidence and cumulative prevalence of EoE has indeed increased more than the rate of annual endoscopies during the observation period[11,12]. This, therefore, argues in favour of a true rise in the incidence and prevalence of the disease.

Attwood *et al*[3] first characterized EoE as a distinct entity from GORD in 1993 where patients with more than 20 eosinophils per high power field and dysphagia in the absence of endoscopic oesophagitis and a normal 24-h pH testing were proposed to have EoE. According to the diagnostic criteria for EoE, other diseases associated with oesophageal eosinophilia must be excluded before a diagnosis of EoE is made (Table 1), with the main differential being GORD[1,13,14]. It is important to distinguish between EoE and GORD as their pathogenesis, natural history, monitoring and treatment differ[15]. This is challenging as many of their clinical and histological features overlap[15,16]. Given the prevalence of GORD in the general population is approximately 20%, it is inevitable that there will be a high probability for EoE to co-exist with GORD[16].

Table 1 Diseases associated with oesophageal eosinophilia

GORD
Eosinophilic gastrointestinal diseases
Atopy
Coeliac disease
Crohn's disease
Oesophageal infections
Hypereosinophilic syndrome
Achalasia
Drug hypersensitivity
Vasculitis
Pemphigoid vegetans
Connective tissue disease
Graft-versus-host-disease
Oesophageal atresia

GORD: Gastro-oesophageal reflux disease.

Prior to the 2017 consensus, a lack of response to a 2-mo course of a proton-pump inhibitor (PPI) was required exclude PPI-responsive oesophageal eosinophilia (PPI-REE) and confirm the diagnosis of EoE[1]. Patients with PPI-REE presented symptomatically like a typical EoE patient, had GORD diagnostically excluded and exhibited a clinicopathologic response to PPI therapy[1]. Recent evidence, however, indicate that differentiating PPI-REE from EoE is counterintuitive as their phenotypic, molecular, mechanistic and therapeutic features cannot be reliably distinguished[15,17-20]. Also, there was no definition regarding the extent of clinical and histological response required to diagnose PPI-REE[13,15]. Thus, the most recent consensus has retracted the term PPI-REE and considers PPI therapy as a therapeutic agent, rather than a diagnostic criterion[5]. The term "PPI-responsive EoE" has been proposed to replace the now defunct PPI-REE[20].

Despites the fact that PPI responders are now considered to be within the EoE continuum, a relationship between EoE and GORD still exists[5]. Studies have suggested that up to 30%-40% of EoE patients may be PPI responsive, either due to a reduction in acid secretion in patients with co-existent GORD or by means of other still unknown anti-inflammatory mechanisms[21,22]. PPI therapy may also be helpful in patients with EoE as the altered oesophagus may be predisposed and more sensitive to acid exposure[23]. This review aims to outline the factors that differentiate between EoE and GORD as well as to evaluate the complex relationship between the two entities in term of pathophysiology and immunology.

PATHOGENESIS

The main pathogenic mechanism of GORD is increased

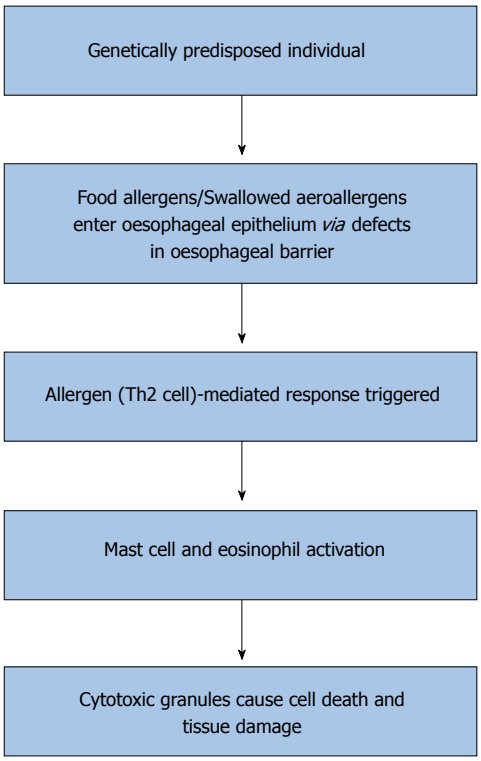

Figure 1 Proposed pathogenesis of eosinophilic oesophagitis.

transient lower oesophageal sphincter (LOS) relaxations (TLOSRs), leading to excessive reflux of gastric acid to the lower oesophageal mucosa[24]. Other potential mechanistic factors that can increase acid reflux to the oesophagus are impaired LOS resting pressure, impaired oesophageal acid clearance, delayed gastric emptying and anatomical factors, such as a hiatus hernia[24]. More recently, impaired mucosal resistance and increased visceral hypersensitivity to acid have also been reported to predispose to GORD[24]. Histologically, it was thought that erosive changes in the distal oesophagus developed due to direct chemical-induced injury of the oesophageal mucosa and death of surface cells[25]. Such injury has been shown to provoke a T-helper Type 1 (Th1) inflammatory response, activating mostly granulocytes and lymphocytes[25]. Thus, it is intriguing that oesophageal eosinophilia can occasionally be seen in GORD, and the underlying mechanism remains unclear[26]. A study showing that GORD may also be a cytokine-mediated disease lead to the discovery that oesophageal squamous cells from EoE and GORD patients exhibit similar levels of eotaxin-3 (a chemokine that attracts eosinophils) when stimulated by T-helper Type 2 (Th2) cytokines; production of which is typical of an allergic disorder[10,15,22,26,27]. This suggests that GORD may be driven to a Th2 inflammatory response when the appropriate stimulus is present leading to oesophageal eosinophilia[26]. Low intraluminal baseline impedance has been shown to be associated with dilatation of intercellular spaces and increased acid exposure in patients with GORD[28]. However, whether this damage can lead to exposure of food allergens and subsequently a Th2 response is unknown[26,29,30].

Although the exact pathophysiology of EoE is not fully understood, substantial evidence exists to show that EoE is an allergen (Th2 cell)-mediated response in genetically predisposed individuals (Figure 1)[10,31,32]. Defects in the oesophageal barrier are thought to facilitate the entry of food allergens or swallowed aeroallergens into the oesophageal epithelium which trigger a Th2 response and lead to mast cell activation and release of mediators such as interleukin (IL)-5, which is a known eosinophil activator[10,22]. Activated eosinophils then release cytotoxic granules which contribute to cell death and tissue damage in these patients[10,33,34]. The gene coding for eotaxin-3, *CCL26* is overexpressed in the oesophagus of patients with EoE compared to healthy controls, which correlates with the increased levels of IL-5 and IL-13 in the oesophagus and blood of EoE patients[35,36]. The development of EoE may also be associated with a genetic predisposition[10]. Hereditary collagen disorders such as Marfan and Ehlers-Danlos syndromes are the most frequent associations of EoE with an incidence of about one percent[21]. In patients with atopic dermatitis, a loss of function mutation in the gene filaggrin (2282del4) is overexpressed in EoE patients compared with healthy controls[37]. Filaggrin is a key structural, keratin-binding protein that plays an important role in the maturation of skin as an epithelial barrier by preventing keratin proteolysis[37]. EoE has been shown in paediatric patients to be associated with variants at chromosome 5q22 encompassing the gene *TSLP* (thymic stromal lymphopoetin), which encodes a cytokine that controls dendritic cell-mediated Th2-cell responses[21,38]. More recently, EoE susceptibility locus was found at 2p23 which encodes *CAPN14*, which is upregulated on exposure to IL-13[39]. However, the exact impact of these genetic abnormalities on the pathogenesis of EoE is uncertain.

EPIDEMIOLOGY AND CLINICAL PRESENTATION

A few epidemiological differences exist between GORD and EoE. GORD is typically diagnosed in the second to fifth decade of life[20]. In contrast, EoE has a bimodal age presentation, with one peak in childhood and the second in the third and fourth decade with the mean age of diagnosis of 38 years[1,33,40]. Furthermore, whilst there is no gender preponderance in GORD, EoE affects males three times more than females[1,41,42]. Both conditions have been more frequently reported in Caucasians compared with other ethnicities[1,8,41,43]. It should be noted that the prevalence of GORD is much higher than that of EoE, ranging between 10%-20% in the Western population as compared to less than 1% for EoE[8,9,40,41]. Obesity has been shown to associated with GORD whereas EoE is strongly associated with atopic diseases

Table 2 Diagnostic features of gastro-oesophageal reflux disease and eosinophilic oesophagitis

	GORD	EoE
Endoscopic	Erosive oesophagitis	Trachealization
	Peptic strictures	Felinization
	Hiatus hernia	Whitish exudates
	Barrett's oesophagus	Longitudinal furrows
		Oedema
		Diffuse oesophageal narrowing
		Narrow-calibre oesophagus
		Oesophageal lacerations
		Loss of mucosal vascular pattern
Histological	Eosinophilia < 10/hpf	Eosinophilia ≥ 15/hpf
		Eosinophilic microabscesses
		Eosinophil degranulation
		Basal cell hyperplasia
		Papillary lengthening
		Superficial layering of eosinophils
		Extracellular eosinophil granules
		Intracytoplasmic keratinocyte vacuolation
		Dilated intracellular spaces
		Lamina propria fibrosis
		Positive intrasqamous IgG4
Motor function	Non-specific	Non-specific

GORD: Gastro-oesophageal reflux disease; EoE: Eosinophilic oesophagitis.

such as asthma, food allergy, eczema, environmental allergies and chronic rhinitis[1,8,10,31,44].

GORD has been defined by the Montreal Classification as a condition that occurs due to retrograde flow of gastric contents into the oesophagus that lead to troublesome symptoms, which are typically heartburn and regurgitation[45,46]. Other less common symptoms include chest pain, dysphagia, dyspepsia, epigastric pain, nausea, bloating, belching, chronic cough, asthma, laryngitis and other respiratory symptoms[45-48]. Whilst dysphagia is infrequent in GORD, it is the most common presenting symptom for EoE along with food bolus impaction[1,10,49]. Approximately 50% of patients who present with food bolus impaction and up to 15% of patients who undergo endoscopy for non-obstructive dysphagia will have EoE[6,50]. Although some EoE patients report GORD symptoms, they may respond poorly to PPIs[51]. Fifty to eighty percent of EoE patients have a prior history of atopic symptoms[21]. Other non-specific symptoms include chest pain, heartburn, regurgitation, dyspepsia, nausea and vomiting, odynophagia, abdominal pain and non-specific throat symptoms[1,10,31,33,49,52].

DIAGNOSIS

A diagnosis of GORD is usually based on clinical symptoms, typically heartburn and regurgitation, in a patient who is responsive to PPI therapy[46]. Thus, upper endoscopy, routine biopsies from the distal oesophagus and ambulatory pH testing are not usually required in a patient with typical GORD symptoms in the absence of alarm symptoms such as dysphagia, odynophagia and weight loss[16,44,46]. The diagnosis of EoE on the other hand, relies on a correlation between clinical symptoms, endoscopic and histological features as there is no one pathognomonic feature of EoE[10,13]. According to the most recent consensus, it requires the presence of ≥ 15 intraepithelial eosinophils per high power field in one or more oesophageal mucosal biopsies in combination with symptoms of oesophageal dysfunction[5]. However, this definition may be too simplified as the diagnosis of EoE may be established with a lower intraepithelial eosinophil count if there is strong clinical suspicion and other histological features associated with eosinophilic inflammation are present[1,10]. Given that excessive accumulation of eosinophils in tissues is a common finding in numerous gastrointestinal disorders, other causes of oesophageal eosinophilia (Table 1) should also be excluded, particularly GORD[1,14]. The following diagnostic features that may be found in GORD and EoE and may help distinguish between the two entities are summarised in Table 2.

Endoscopic oesophageal features

Relevant endoscopic findings of GORD are erosive oesophagitis, peptic strictures, a hiatus hernia and Barrett's oesophagus[15,16,46]. Endoscopy has a high specificity for diagnosing GORD particularly when erosive oesophagitis is seen and the Los Angeles classification is used[53]. However, most patients with GORD will have normal endoscopies[15,16]. In contrast, endoscopic oesophageal features of EoE patients are trachealization, felinization, whitish exudates, longitudinal furrows, oedema, diffuse oesophageal narrowing, narrow-calibre oesophagus and oesophageal lacerations secondary to passage of the endoscope[1,10,13,16,54] (Figure 2). Loss of

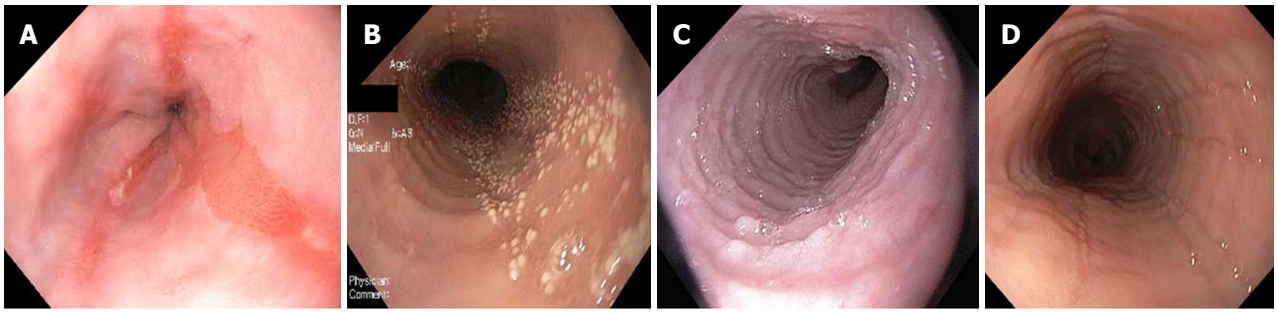

Figure 2 Endoscopic changes in patients with gastro-oesophageal reflux disease and eosinophilic oesophagitis. A: Erosive oesophagitis of gastro-oesophageal reflux disease; B: White exudates in eosinophilic oesophagitis (EoE); C: Mucosal rings or trachealization in EoE; D: Longitudinal furrows in EoE.

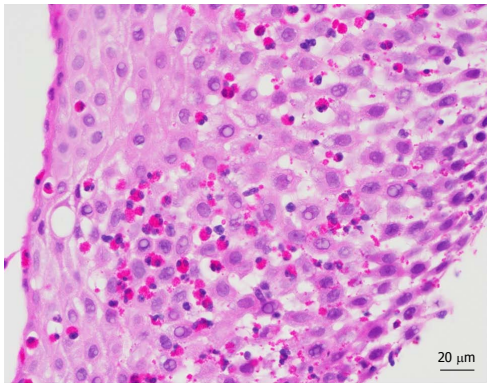

Figure 3 Histological specimen from the oesophagus (luminal aspect on left) of an eosinophilic oesophagitis patient showing marked oedema and numerous intraepithelial eosinophils in the oesophageal squamous mucosa, which are also seen in the superficial component of the mucosa.

mucosal vascular pattern has also been reported[55]. These features however, are not pathognomonic for EoE and thus histological correlation is required[1,10]. Normal endoscopic findings have been reported in up to 30% of patients with EoE[10,13].

Histological features

Patients with GORD may exhibit oesophageal eosinophilia, typically less than 10 per high power field as compared to ≥ 15 per high power field for EoE[1,10,15,56] (Figure 3). The presence of additional histological features of eosinophilic microabscesses, eosinophil degranulation, basal cell hyperplasia, papillary lengthening, superficial layering of eosinophils, extracellular eosinophil granules, intracytoplasmic keratinocyte vacuolation, dilated intracellular spaces or lamina propria fibrosis are more supportive of a diagnosis of EoE[1,10,13,16,57]. Although some of these additional histological features have been reported in biopsy specimens of patients with GORD, they are less commonly found as compared to EoE[10,13,16,57]. Recently, Zukerberg *et al*[17] showed that immunohistochemical staining of oesophageal tissue with IgG4 could help distinguish EoE from GORD, given that 76% of EoE cases were positive for intrasqamous IgG4 and none of the GORD cases were positive. The distribution of oesophageal eosinophilia may also be helpful in distinguishing the two conditions, with diffuse oesophageal eosinophilia more suggestive of EoE and distal oesophageal eosinophilia of GORD[16]. Thus, it is important to biopsy at least 2 regions of the oesophagus and accurately label the site of oesophageal biopsies.

Oesophageal motor function

Oesophageal manometry is of limited use in the diagnosis of GORD and EoE given that findings have so far been non-specific[1,13,58]. Oesophageal motility disorders found in patients with GORD have a similar type and prevalence to patients with EoE ranging between 4%-87%[14,21,33]. However, in cases where dysphagia is the main symptom, it is important to perform manometric assessment to exclude major and minor disorders of peristalsis which can sometimes mimic symptoms of GORD and EoE[18,33]. The duration of EoE has been shown to be longer in those with abnormal oesophageal motility[59].

TREATMENT

The initial management of GORD usually involves a combination of lifestyle interventions and medical therapy with the aim of eliminating symptoms, repairing any existing oesophageal mucosal injury and preventing further inflammatory injury[46,60]. Lifestyle interventions of weight loss (particularly if BMI > 25 or recent weight gain) and head of bed elevation have been proven to reduce symptoms and improve oesophageal pH values[61,62]. Other lifestyle interventions such as avoidance of late evening meals and cessation of alcohol, tobacco, chocolate, caffeine, spicy foods, citrus and carbonated drinks lack evidence and are not routinely recommended[46]. Medical therapy such as antacids, histamine-receptor antagonists (H_2RA) or PPI therapy should then be considered in patients failing lifestyle interventions alone[46,60]. PPI therapy is effective in 70%-80% of patients and has been shown to be superior to H_2RAs in regard to healing rates and decreased relapse rates[63]. Surgical therapy is as effective as medical therapy and may be contemplated in GORD patients who wish to discontinue medications, are non-compliant, have side-effects associated with medications, have a

large hiatus hernia or have refractory oesophagitis and symptoms despite optimal medical therapy[46].

The choice of initial treatment for EoE patients on the other hand is made on an individualized basis as PPI therapy, topical steroids and dietary therapy can all be considered as first-line therapeutic options[5]. All EoE patients should receive treatment to improve quality of life, prevent oesophageal remodelling secondary to active eosinophilic inflammation and prevent oesophageal injury due to the disease or endoscopic intervention[64]. 30%-40% of EoE patients may be responsive to PPIs, either due to a reduction in acid secretion in patients with co-existent GORD or by means of other still unknown anti-inflammatory mechanisms[21,22]. EoE patients can also be treated with topical steroids as it has been shown to improve symptoms and reduces oesophageal eosinophilia[21,65]. Viscous steroids have been shown to be more effective than nebulized steroids possibly due to greater mucosal contact time compared with the latter[66]. A recent meta-analysis of seven randomized controlled trials concluded that although there was an increased risk of asymptomatic oesophageal candidiasis with topical steroid therapy, it is considered safe with no evidence of adrenal suppression[67]. Dietary therapy is based on the fact that the majority of EoE patients have food allergies that may contribute to the pathogenesis of the disease[22,68]. There are 3 strategies of dietary therapy: An amino acid-based formula/elemental diet, a targeted elimination diet guided by allergy testing, and an empiric elimination diet[22,65,68]. All diets should be followed for a minimum of 6 wk and its efficacy evaluated *via* symptoms as well and oesophageal biopsies[65,69].

Oesophageal dilation, either *via* through-the-scope balloons or by Savary bougies can lead to long-lasting symptom improvement in EoE patients with structuring disease or impaired oesophageal distensibility due to subepithelial fibrosis[21,22]. Clinical improvement post dilation occurred in 75% of patients[70]. A meta-analysis evaluating the clinical efficacy and safety of oesophageal dilation in these patients showed that it is a safe procedure with a < 1% rate of serious complications[70]. However, it does not result in a decreased in eosinophil infiltration or histologic improvement and thus should not be used as a sole therapeutic option in these patients[5,71]. Several other treatment options for EoE have been assessed namely Montelukast (leukotriene receptor antagonist), Infliximab (anti-tumour necrosis factor), Mepolizumab (anti-IL-5), Azathioprine or 6-mercaptopurine, Reslizumab (IL-5 neutralizing antibody), Omalizumab (anti-IgE), QAX576 (anti-IL-13) and OC000459 (prostaglandin D2 receptor antagonist)[34,64,72-80] Although studies of these agents have shown changes in the biological behaviour of EoE disease markers, they have not yet displayed sufficient clinical benefit for widespread use[81].

RELATIONSHIP BETWEEN EoE AND GASTROESOPHAGEAL REFLUX DISEASE

The interaction between EoE and GORD is complex and may be bidirectional[5]. An approximate prevalence of GORD in the general population of 20% is sufficiently high enough to make the coexistence of EoE and GORD plausible[16]. In patients with refractory GORD symptoms, EoE was found in approximately 4%[10,56]. Four hypotheses to account for interactions between oesophageal eosinophilia and GORD have been proposed: Eosinophilia as a marker of GORD; GORD and EoE coexist but are unrelated, EoE contributes or causes GORD; and GORD contributes to or causes EoE[16,20,82,83].

Eosinophilia as a marker of GORD

GORD is thought to cause a mild eosinophilia in the absence of EoE[16,82]. Acid exposure was thought to cause oesophageal injury which results in chronic inflammation, including the presence of oesophageal eosinophils that are recruited *via* an increase in expression of adhesion molecules, release of chemokines that attract eosinophils and increase in blood flow[16]. However, the role of these adhesion molecules and chemokines in the pathogenesis of GORD is yet unclear[16]. A study also showed that dense oesophageal eosinophilia in GORD was uncommon[3].

GORD and EoE coexist but are unrelated

As mentioned above, due to a high prevalence of GORD in the general population, the coexistence of EoE and GORD due to chance alone is plausible[16,83]. Oesophageal pH studies have shown that 25%-50% of EoE patients have increased oesophageal acid exposure, thus supporting the notion that the two entities can coexist[1,16].

EoE contributes or causes GORD

This hypothesis is based on the fact that eosinophils secrete a number of agents that affect the integrity of the mucosal barrier and the function of oesophageal smooth muscle as well as producing a direct cytotoxic effect on the mucosa[16,20]. Remodelling effect in EoE may contribute to increased acid exposure due to effects on the LOS or impaired oesophageal clearance of refluxed contents[16,20].

GORD contributes to or causes EoE

An unproven hypothesis has suggested that GORD may contribute to the pathogenesis of EoE by causing changes in the integrity of the oesophageal mucosa, promoting trans-epithelial allergen permeation followed by allergic immune activation[5,84].

CONCLUSION

The relationship between EoE and GORD is complex as

they are different entities that may coexist. Distinguishing between the two remains challenging given that it has multiple overlapping features. At present, the combination of clinical, endoscopic and histological features, as well as response to PPI therapy, may help to differentiate the two conditions. Further studies into the immunopathophysiology are needed to elucidate more objective diagnostic testing that can reliably differentiate between the two disease processes.

REFERENCES

1 **Liacouras CA**, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA, Burks AW, Chehade M, Collins MH, Dellon ES, Dohil R, Falk GW, Gonsalves N, Gupta SK, Katzka DA, Lucendo AJ, Markowitz JE, Noel RJ, Odze RD, Putnam PE, Richter JE, Romero Y, Ruchelli E, Sampson HA, Schoepfer A, Shaheen NJ, Sicherer SH, Spechler S, Spergel JM, Straumann A, Wershil BK, Rothenberg ME, Aceves SS. Eosinophilic esophagitis: updated consensus recommendations for children and adults. *J Allergy Clin Immunol* 2011; **128**: 3-20.e6; quiz 21-22 [PMID: 21477849 DOI: 10.1016/j.jaci.2011.02.040]

2 **Schreiber MH**. Granuloma of the esophagogastric junction with eosinophilic infiltration. *Gastroenterology* 1962; **43**: 206-211 [PMID: 13909260]

3 **Attwood SE**, Smyrk TC, Demeester TR, Jones JB. Esophageal eosinophilia with dysphagia. A distinct clinicopathologic syndrome. *Dig Dis Sci* 1993; **38**: 109-116 [PMID: 8420741 DOI: 10.1007/BF01296781]

4 **Furuta GT**, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, Bonis P, Hassall E, Straumann A, Rothenberg ME; First International Gastrointestinal Eosinophil Research Symposium (FIGERS) Subcommittees. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. *Gastroenterology* 2007; **133**: 1342-1363 [PMID: 17919504 DOI: 10.1053/j.gastro.2007.08.017]

5 **Lucendo AJ**, Molina-Infante J, Arias Á, von Arnim U, Bredenoord AJ, Bussmann C, Amil Dias J, Bove M, González-Cervera J, Larsson H, Miehlke S, Papadopoulou A, Rodríguez-Sánchez J, Ravelli A, Ronkainen J, Santander C, Schoepfer AM, Storr MA, Terreehorst I, Straumann A, Attwood SE. Guidelines on eosinophilic esophagitis: evidence-based statements and recommendations for diagnosis and management in children and adults. *United European Gastroenterol J* 2017; **5**: 335-358 [PMID: 28507746 DOI: 10.1177/2050640616689525]

6 **Arias Á**, Pérez-Martínez I, Tenías JM, Lucendo AJ. Systematic review with meta-analysis: the incidence and prevalence of eosinophilic oesophagitis in children and adults in population-based studies. *Aliment Pharmacol Ther* 2016; **43**: 3-15 [PMID: 26510832 DOI: 10.1111/apt.13441]

7 **Shaheen NJ**, Mukkada V, Eichinger CS, Schofield H, Todorova L, Falk GW. Natural history of eosinophilic esophagitis: a systematic review of epidemiology and disease course. *Dis Esophagus* 2018; **31** [PMID: 29617744 DOI: 10.1093/dote/doy015]

8 **Dellon ES**. Epidemiology of eosinophilic esophagitis. *Gastroenterol Clin North Am* 2014; **43**: 201-218 [PMID: 24813510 DOI: 10.1016/j.gtc.2014.02.002]

9 **Mahesh VN**, Holloway RH, Nguyen NQ. Changing epidemiology of food bolus impaction: is eosinophilic esophagitis to blame? *J Gastroenterol Hepatol* 2013; **28**: 963-966 [PMID: 23425056 DOI: 10.1111/jgh.12135]

10 **Ali MA**, Lam-Himlin D, Voltaggio L. Eosinophilic esophagitis: a clinical, endoscopic, and histopathologic review. *Gastrointest Endosc* 2012; **76**: 1224-1237 [PMID: 23025974 DOI: 10.1016/j.gie.2012.08.023]

11 **Straumann A**, Spichtin HP, Grize L, Bucher KA, Beglinger C, Simon HU. Natural history of primary eosinophilic esophagitis: a follow-up of 30 adult patients for up to 11.5 years. *Gastroenterology* 2003; **125**: 1660-1669 [PMID: 14724818 DOI: 10.1053/j.gastro.2003.09.024]

12 **Giriens B**, Yan P, Safroneeva E, Zwahlen M, Reinhard A, Nydegger A, Vavricka S, Sempoux C, Straumann A, Schoepfer AM. Escalating incidence of eosinophilic esophagitis in Canton of Vaud, Switzerland, 1993-2013: a population-based study. *Allergy* 2015; **70**: 1633-1639 [PMID: 26304142 DOI: 10.1111/all.12733]

13 **Schoepfer A**. Diagnostic approach to eosinophilic oesophagitis: Pearls and pitfalls. *Best Pract Res Clin Gastroenterol* 2015; **29**: 783-792 [PMID: 26552777 DOI: 10.1016/j.bpg.2015.06.014]

14 **Lucendo AJ**. Disease associations in eosinophilic oesophagitis and oesophageal eosinophilia. *Best Pract Res Clin Gastroenterol* 2015; **29**: 759-769 [PMID: 26552775 DOI: 10.1016/j.bpg.2015.06.010]

15 **Molina-Infante J**, Bredenoord AJ, Cheng E, Dellon ES, Furuta GT, Gupta SK, Hirano I, Katzka DA, Moawad FJ, Rothenberg ME, Schoepfer A, Spechler SJ, Wen T, Straumann A, Lucendo AJ; PPI-REE Task Force of the European Society of Eosinophilic Oesophagitis (EUREOS). Proton pump inhibitor-responsive oesophageal eosinophilia: an entity challenging current diagnostic criteria for eosinophilic oesophagitis. *Gut* 2016; **65**: 524-531 [PMID: 26685124 DOI: 10.1136/gutjnl-2015-310991]

16 **Kia L**, Hirano I. Distinguishing GERD from eosinophilic oesophagitis: concepts and controversies. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 379-386 [PMID: 25986303 DOI: 10.1038/nrgastro.2015.75]

17 **Zukerberg L**, Mahadevan K, Selig M, Deshpande V. Oesophageal intrasquamous IgG4 deposits: an adjunctive marker to distinguish eosinophilic oesophagitis from reflux oesophagitis. *Histopathology* 2016; **68**: 968-976 [PMID: 26466342 DOI: 10.1111/his.12892]

18 **Santander C**, Chavarría-Herbozo CM, Becerro-González I, Burgos-Santamaría D. Impaired esophageal motor function in eosinophilic esophagitis. *Rev Esp Enferm Dig* 2015; **107**: 622-629 [PMID: 26437981 DOI: 10.17235/reed.2015.3801/2015]

19 **Jiao D**, Ishimura N, Maruyama R, Ishikawa N, Nagase M, Oshima N, Aimi M, Okimoto E, Mikami H, Izumi D, Okada M, Ishihara S, Kinoshita Y. Similarities and differences among eosinophilic esophagitis, proton-pump inhibitor-responsive esophageal eosinophilia, and reflux esophagitis: comparisons of clinical, endoscopic, and histopathological findings in Japanese patients. *J Gastroenterol* 2017; **52**: 203-210 [PMID: 27108416 DOI: 10.1007/s00535-016-1213-1]

20 **Molina-Infante J**, van Rhijn BD. Interactions between gastro-oesophageal reflux disease and eosinophilic oesophagitis. *Best Pract Res Clin Gastroenterol* 2015; **29**: 749-758 [PMID: 26552774 DOI: 10.1016/j.bpg.2015.06.009]

21 **Guarino MP**, Cicala M, Behar J. Eosinophilic esophagitis: New insights in pathogenesis and therapy. *World J Gastrointest Pharmacol Ther* 2016; **7**: 66-77 [PMID: 26855813 DOI: 10.4292/wjgpt.v7.i1.66]

22 **Schoepfer A**, Safroneeva E, Straumann A. Eosinophilic Esophagitis: Impact of Latest Insights Into Pathophysiology on Therapeutic Strategies. *Dig Dis* 2016; **34**: 462-468 [PMID: 27333298 DOI: 10.1159/000445201]

23 **Krarup AL**, Villadsen GE, Mejlgaard E, Olesen SS, Drewes AM, Funch-Jensen P. Acid hypersensitivity in patients with eosinophilic oesophagitis. *Scand J Gastroenterol* 2010; **45**: 273-281 [PMID: 20001646 DOI: 10.3109/00365520903469931]

24 **De Giorgi F**, Palmiero M, Esposito I, Mosca F, Cuomo R. Pathophysiology of gastro-oesophageal reflux disease. *Acta Otorhinolaryngol Ital* 2006; **26**: 241-246 [PMID: 17345925]

25 **Molina-Infante J**, Gonzalez-Cordero PL, Lucendo AJ. Proton pump inhibitor-responsive esophageal eosinophilia: still a valid diagnosis? *Curr Opin Gastroenterol* 2017; **33**: 285-292 [PMID: 28445189 DOI: 10.1097/MOG.0000000000000371]

26 **Cheng E**, Zhang X, Huo X, Yu C, Zhang Q, Wang DH, Spechler SJ, Souza RF. Omeprazole blocks eotaxin-3 expression by

oesophageal squamous cells from patients with eosinophilic oesophagitis and GORD. *Gut* 2013; **62**: 824-832 [PMID: 22580413 DOI: 10.1136/gutjnl-2012-302250]

27 **Dunbar KB**, Agoston AT, Odze RD, Huo X, Pham TH, Cipher DJ, Castell DO, Genta RM, Souza RF, Spechler SJ. Association of Acute Gastroesophageal Reflux Disease With Esophageal Histologic Changes. *JAMA* 2016; **315**: 2104-2112 [PMID: 27187303 DOI: 10.1001/jama.2016.5657]

28 **Kandulski A**, Weigt J, Caro C, Jechorek D, Wex T, Malfertheiner P. Esophageal intraluminal baseline impedance differentiates gastroesophageal reflux disease from functional heartburn. *Clin Gastroenterol Hepatol* 2015; **13**: 1075-1081 [PMID: 25496815 DOI: 10.1016/j.cgh.2014.11.033]

29 **Tobey NA**, Carson JL, Alkiek RA, Orlando RC. Dilated intercellular spaces: a morphological feature of acid reflux--damaged human esophageal epithelium. *Gastroenterology* 1996; **111**: 1200-1205 [PMID: 8898633 DOI: 10.1053/gast.1996.v111.pm8898633]

30 **Katzka DA**. The complex relationship between eosinophilic esophagitis and gastroesophageal reflux disease. *Dig Dis* 2014; **32**: 93-97 [PMID: 24603388 DOI: 10.1159/000357080]

31 **Remedios M**, Campbell C, Jones DM, Kerlin P. Eosinophilic esophagitis in adults: clinical, endoscopic, histologic findings, and response to treatment with fluticasone propionate. *Gastrointest Endosc* 2006; **63**: 3-12 [PMID: 16377308 DOI: 10.1016/j.gie.2005.07.049]

32 **Ronkainen J**, Talley NJ, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, Vieth M, Stolte M, Walker MM, Agréus L. Prevalence of oesophageal eosinophils and eosinophilic oesophagitis in adults: the population-based Kalixanda study. *Gut* 2007; **56**: 615-620 [PMID: 17135307 DOI: 10.1136/gut.2006.107714]

33 **Weiss AH**, Iorio N, Schey R. Esophageal motility in eosinophilic esophagitis. *Rev Gastroenterol Mex* 2015; **80**: 205-213 [PMID: 26275635 DOI: 10.1016/j.rgmx.2015.05.005]

34 **Straumann A**. Eosinophilic esophagitis: emerging therapies and future perspectives. *Gastroenterol Clin North Am* 2014; **43**: 385-394 [PMID: 24813523 DOI: 10.1016/j.gtc.2014.02.005]

35 **Blanchard C**, Stucke EM, Rodriguez-Jimenez B, Burwinkel K, Collins MH, Ahrens A, Alexander ES, Butz BK, Jameson SC, Kaul A, Franciosi JP, Kushner JP, Putnam PE, Abonia JP, Rothenberg ME. A striking local esophageal cytokine expression profile in eosinophilic esophagitis. *J Allergy Clin Immunol* 2011; **127**: 208-217, 217.e1-217.e7 [PMID: 21211656 DOI: 10.1016/j.jaci.2010.10.039]

36 **Blanchard C**, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, Jameson SC, Kirby C, Konikoff MR, Collins MH, Cohen MB, Akers R, Hogan SP, Assa'ad AH, Putnam PE, Aronow BJ, Rothenberg ME. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J Clin Invest* 2006; **116**: 536-547 [PMID: 16453027 DOI: 10.1172/JCI26679]

37 **Kawasaki H**, Nagao K, Kubo A, Hata T, Shimizu A, Mizuno H, Yamada T, Amagai M. Altered stratum corneum barrier and enhanced percutaneous immune responses in filaggrin-null mice. *J Allergy Clin Immunol* 2012; **129**: 1538-46.e6 [PMID: 22409988 DOI: 10.1016/j.jaci.2012.01.068]

38 **Blanchard C**, Stucke EM, Burwinkel K, Caldwell JM, Collins MH, Ahrens A, Buckmeier BK, Jameson SC, Greenberg A, Kaul A, Franciosi JP, Kushner JP, Martin LJ, Putnam PE, Abonia JP, Wells SI, Rothenberg ME. Coordinate interaction between IL-13 and epithelial differentiation cluster genes in eosinophilic esophagitis. *J Immunol* 2010; **184**: 4033-4041 [PMID: 20208004 DOI: 10.4049/jimmunol.0903069]

39 **Kottyan LC**, Davis BP, Sherrill JD, Liu K, Rochman M, Kaufman K, Weirauch MT, Vaughn S, Lazaro S, Rupert AM, Kohram M, Stucke EM, Kemme KA, Magnusen A, He H, Dexheimer P, Chehade M, Wood RA, Pesek RD, Vickery BP, Fleischer DM, Lindbad R, Sampson HA, Mukkada VA, Putnam PE, Abonia JP, Martin LJ, Harley JB, Rothenberg ME. Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease. *Nat Genet* 2014; **46**: 895-900 [PMID: 25017104 DOI: 10.1038/ng.3033]

40 **Kapel RC**, Miller JK, Torres C, Aksoy S, Lash R, Katzka DA. Eosinophilic esophagitis: a prevalent disease in the United States that affects all age groups. *Gastroenterology* 2008; **134**: 1316-1321 [PMID: 18471509 DOI: 10.1053/j.gastro.2008.02.016]

41 **El-Serag HB**, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* 2014; **63**: 871-880 [PMID: 23853213 DOI: 10.1136/gutjnl-2012-304269]

42 **Dent J**, El-Serag HB, Wallander MA, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* 2005; **54**: 710-717 [PMID: 15831922 DOI: 10.1136/gut.2004.051821]

43 **Gonsalves N**. Distinct features in the clinical presentations of eosinophilic esophagitis in children and adults: is this the same disease? *Dig Dis* 2014; **32**: 89-92 [PMID: 24603387 DOI: 10.1159/000357078]

44 **Antunes C**, Curtis SA. Gastroesophageal Reflux Disease. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2018 [PMID: 28722967]

45 **Vakil N**, van Zanten SV, Kahrilas P, Dent J, Jones R; Global Consensus Group. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am J Gastroenterol* 2006; **101**: 1900-1920; quiz 1943 [PMID: 16928254 DOI: 10.1111/j.1572-0241.2006.00630.x]

46 **Katz PO**, Gerson LB, Vela MF. Guidelines for the diagnosis and management of gastroesophageal reflux disease. *Am J Gastroenterol* 2013; **108**: 308-28; quiz 329 [PMID: 23419381 DOI: 10.1038/ajg.2012.444]

47 **Gerson LB**, Kahrilas PJ, Fass R. Insights into gastroesophageal reflux disease-associated dyspeptic symptoms. *Clin Gastroenterol Hepatol* 2011; **9**: 824-833 [PMID: 21699806 DOI: 10.1016/j.cgh.2011.05.015]

48 **Badillo R**, Francis D. Diagnosis and treatment of gastroesophageal reflux disease. *World J Gastrointest Pharmacol Ther* 2014; **5**: 105-112 [PMID: 25133039 DOI: 10.4292/wjgpt.v5.i3.105]

49 **Sá CC**, Kishi HS, Silva-Werneck AL, Moraes-Filho JP, Eisig JN, Barbuti RC, Hashimoto CL, Navarro-Rodriguez T. Eosinophilic esophagitis in patients with typical gastroesophageal reflux disease symptoms refractory to proton pump inhibitor. *Clinics* (Sao Paulo) 2011; **66**: 557-561 [PMID: 21655746]

50 **Kerlin P**, Jones D, Remedios M, Campbell C. Prevalence of eosinophilic esophagitis in adults with food bolus obstruction of the esophagus. *J Clin Gastroenterol* 2007; **41**: 356-361 [PMID: 17413601 DOI: 10.1097/01.mcg.0000225590.08825.77]

51 **Menard-Katcher P**, Marks KL, Liacouras CA, Spergel JM, Yang YX, Falk GW. The natural history of eosinophilic oesophagitis in the transition from childhood to adulthood. *Aliment Pharmacol Ther* 2013; **37**: 114-121 [PMID: 23121227 DOI: 10.1111/apt.12119]

52 **Kinoshita Y**, Furuta K, Ishimaura N, Ishihara S, Sato S, Maruyama R, Ohara S, Matsumoto T, Sakamoto C, Matsui T, Ishikawa S, Chiba T. Clinical characteristics of Japanese patients with eosinophilic esophagitis and eosinophilic gastroenteritis. *J Gastroenterol* 2013; **48**: 333-339 [PMID: 22847555 DOI: 10.1007/s00535-012-0640-x]

53 **Lundell LR**, Dent J, Bennett JR, Blum AL, Armstrong D, Galmiche JP, Johnson F, Hongo M, Richter JE, Spechler SJ, Tytgat GN, Wallin L. Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. *Gut* 1999; **45**: 172-180 [PMID: 10403727 DOI: 10.1136/gut.45.2.172]

54 **Potter JW**, Saeian K, Staff D, Massey BT, Komorowski RA, Shaker R, Hogan WJ. Eosinophilic esophagitis in adults: an emerging problem with unique esophageal features. *Gastrointest Endosc* 2004; **59**: 355-361 [PMID: 14997131 DOI: 10.1016/

S0016-5107(03)02713-5]
55 **Straumann A**, Spichtin HP, Bucher KA, Heer P, Simon HU. Eosinophilic esophagitis: red on microscopy, white on endoscopy. *Digestion* 2004; **70**: 109-116 [PMID: 15383737 DOI: 10.1159/000080934]
56 **García-Compeán D**, González González JA, Marrufo García CA, Flores Gutiérrez JP, Barboza Quintana O, Galindo Rodríguez G, Mar Ruiz MA, de León Valdez D, Jaquez Quintana JO, Maldonado Garza HJ. Prevalence of eosinophilic esophagitis in patients with refractory gastroesophageal reflux disease symptoms: A prospective study. *Dig Liver Dis* 2011; **43**: 204-208 [PMID: 20843755 DOI: 10.1016/j.dld.2010.08.002]
57 **Naik RD**, Vaezi MF. Recent advances in diagnostic testing for gastroesophageal reflux disease. *Expert Rev Gastroenterol Hepatol* 2017; **11**: 531-537 [PMID: 28317452 DOI: 10.1080/17474124.2017.1309286]
58 **Kahrilas PJ**, Shaheen NJ, Vaezi MF, Hiltz SW, Black E, Modlin IM, Johnson SP, Allen J, Brill JV; American Gastroenterological Association. American Gastroenterological Association Medical Position Statement on the management of gastroesophageal reflux disease. *Gastroenterology* 2008; **135**: 1383-1391, 1391.e1-1391.e5 [PMID: 18789939 DOI: 10.1053/j.gastro.2008.08.045]
59 **van Rhijn BD**, Oors JM, Smout AJ, Bredenoord AJ. Prevalence of esophageal motility abnormalities increases with longer disease duration in adult patients with eosinophilic esophagitis. *Neurogastroenterol Motil* 2014; **26**: 1349-1355 [PMID: 25039642 DOI: 10.1111/nmo.12400]
60 **Patti MG**. An Evidence-Based Approach to the Treatment of Gastroesophageal Reflux Disease. *JAMA Surg* 2016; **151**: 73-78 [PMID: 26629969 DOI: 10.1001/jamasurg.2015.4233]
61 **Pollmann H**, Zillessen E, Pohl J, Rosemeyer D, Abucar A, Armbrecht U, Bornhofen B, Herz R. Effect of elevated head position in bed in therapy of gastroesophageal reflux. *Z Gastroenterol* 1996; **34** Suppl 2: 93-99 [PMID: 8767437]
62 **Jacobson BC**, Somers SC, Fuchs CS, Kelly CP, Camargo CA Jr. Body-mass index and symptoms of gastroesophageal reflux in women. *N Engl J Med* 2006; **354**: 2340-2348 [PMID: 16738270 DOI: 10.1056/NEJMoa054391]
63 **Labenz J**, Malfertheiner P. Treatment of uncomplicated reflux disease. *World J Gastroenterol* 2005; **11**: 4291-4299 [PMID: 16038023 DOI: 10.3748/wjg.v11.i28.4291]
64 **Straumann A**. Medical therapy in eosinophilic oesophagitis. *Best Pract Res Clin Gastroenterol* 2015; **29**: 805-814 [PMID: 26552779 DOI: 10.1016/j.bpg.2015.06.012]
65 **Dellon ES**, Gonsalves N, Hirano I, Furuta GT, Liacouras CA, Katzka DA; American College of Gastroenterology. ACG clinical guideline: Evidenced based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis (EoE). *Am J Gastroenterol* 2013; **108**: 679-692; quiz 693 [PMID: 23567357 DOI: 10.1038/ajg.2013.71]
66 **Dellon ES**, Sheikh A, Speck O, Woodward K, Whitlow AB, Hores JM, Ivanovic M, Chau A, Woosley JT, Madanick RD, Orlando RC, Shaheen NJ. Viscous topical is more effective than nebulized steroid therapy for patients with eosinophilic esophagitis. *Gastroenterology* 2012; **143**: 321-324.e1 [PMID: 22561055 DOI: 10.1053/j.gastro.2012.04.049]
67 **Chuang MY**, Chinnaratha MA, Hancock DG, Woodman R, Wong GR, Cock C, Fraser RJ. Topical Steroid Therapy for the Treatment of Eosinophilic Esophagitis (EoE): A Systematic Review and Meta-Analysis. *Clin Transl Gastroenterol* 2015; **6**: e82 [PMID: 25809314 DOI: 10.1038/ctg.2015.9]
68 **Warners MJ**, Vlieg-Boerstra BJ, Bredenoord AJ. Elimination and elemental diet therapy in eosinophilic oesophagitis. *Best Pract Res Clin Gastroenterol* 2015; **29**: 793-803 [PMID: 26552778 DOI: 10.1016/j.bpg.2015.06.013]
69 **Molina-Infante J**, Gonzalez-Cordero PL, Arias A, Lucendo AJ. Update on dietary therapy for eosinophilic esophagitis in children and adults. *Expert Rev Gastroenterol Hepatol* 2017; **11**: 115-123 [PMID: 27998193 DOI: 10.1080/17474124.2017.1271324]
70 **Moawad FJ**, Cheatham JG, DeZee KJ. Meta-analysis: the safety and efficacy of dilation in eosinophilic oesophagitis. *Aliment Pharmacol Ther* 2013; **38**: 713-720 [PMID: 23915046 DOI: 10.1111/apt.12438]
71 **Schoepfer AM**, Gonsalves N, Bussmann C, Conus S, Simon HU, Straumann A, Hirano I. Esophageal dilation in eosinophilic esophagitis: effectiveness, safety, and impact on the underlying inflammation. *Am J Gastroenterol* 2010; **105**: 1062-1070 [PMID: 19935783 DOI: 10.1038/ajg.2009.657]
72 **Attwood SE**, Lewis CJ, Bronder CS, Morris CD, Armstrong GR, Whittam J. Eosinophilic oesophagitis: a novel treatment using Montelukast. *Gut* 2003; **52**: 181-185 [PMID: 12524397 DOI: 10.1136/gut.52.2.181]
73 **Lucendo AJ**, De Rezende LC, Jiménez-Contreras S, Yagüe-Compadre JL, González-Cervera J, Mota-Huertas T, Guagnozzi D, Angueira T, González-Castillo S, Arias A. Montelukast was inefficient in maintaining steroid-induced remission in adult eosinophilic esophagitis. *Dig Dis Sci* 2011; **56**: 3551-3558 [PMID: 21674173 DOI: 10.1007/s10620-011-1775-y]
74 **Straumann A**, Bussmann C, Conus S, Beglinger C, Simon HU. Anti-TNF-alpha (infliximab) therapy for severe adult eosinophilic esophagitis. *J Allergy Clin Immunol* 2008; **122**: 425-427 [PMID: 18678345 DOI: 10.1016/j.jaci.2008.06.012]
75 **Assa'ad AH**, Gupta SK, Collins MH, Thomson M, Heath AT, Smith DA, Perschy TL, Jurgensen CH, Ortega HG, Aceves SS. An antibody against IL-5 reduces numbers of esophageal intraepithelial eosinophils in children with eosinophilic esophagitis. *Gastroenterology* 2011; **141**: 1593-1604 [PMID: 21835135 DOI: 10.1053/j.gastro.2011.07.044]
76 **Straumann A**, Conus S, Grzonka P, Kita H, Kephart G, Bussmann C, Beglinger C, Smith DA, Patel J, Byrne M, Simon HU. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. *Gut* 2010; **59**: 21-30 [PMID: 19828470 DOI: 10.1136/gut.2009.178558]
77 **Netzer P**, Gschossmann JM, Straumann A, Sendensky A, Weimann R, Schoepfer AM. Corticosteroid-dependent eosinophilic oesophagitis: azathioprine and 6-mercaptopurine can induce and maintain long-term remission. *Eur J Gastroenterol Hepatol* 2007; **19**: 865-869 [PMID: 17873610 DOI: 10.1097/MEG.0b013e32825a6ab4]
78 **Spergel JM**, Rothenberg ME, Collins MH, Furuta GT, Markowitz JE, Fuchs G 3rd, O'Gorman MA, Abonia JP, Young J, Henkel T, Wilkins HJ, Liacouras CA. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2012; **129**: 456-463, 463.e1-463.e3 [PMID: 22206777 DOI: 10.1016/j.jaci.2011.11.044]
79 **Rocha R**, Vitor AB, Trindade E, Lima R, Tavares M, Lopes J, Dias JA. Omalizumab in the treatment of eosinophilic esophagitis and food allergy. *Eur J Pediatr* 2011; **170**: 1471-1474 [PMID: 21809010 DOI: 10.1007/s00431-011-1540-4]
80 **Straumann A**, Hoesli S, Bussmann Ch, Stuck M, Perkins M, Collins LP, Payton M, Pettipher R, Hunter M, Steiner J, Simon HU. Anti-eosinophil activity and clinical efficacy of the CRTH2 antagonist OC000459 in eosinophilic esophagitis. *Allergy* 2013; **68**: 375-385 [PMID: 23379537 DOI: 10.1111/all.12096]
81 **Attwood S**, Sabri S. Historical aspects of eosinophilic esophagitis: from case reports to clinical trials. *Dig Dis* 2014; **32**: 34-39 [PMID: 24603378 DOI: 10.1159/000357007]
82 **Cheng E**, Souza RF, Spechler SJ. Eosinophilic esophagitis: interactions with gastroesophageal reflux disease. *Gastroenterol Clin North Am* 2014; **43**: 243-256 [PMID: 24813513 DOI: 10.1016/j.gtc.2014.02.004]
83 **Spechler SJ**, Genta RM, Souza RF. Thoughts on the complex relationship between gastroesophageal reflux disease and eosinophilic esophagitis. *Am J Gastroenterol* 2007; **102**: 1301-1306

[PMID: 17531015 DOI: 10.1111/j.1572-0241.2007.01179.x]

84 **van Rhijn BD**, Weijenborg PW, Verheij J, van den Bergh Weerman MA, Verseijden C, van den Wijngaard RM, de Jonge WJ, Smout AJ, Bredenoord AJ. Proton pump inhibitors partially restore mucosal integrity in patients with proton pump inhibitor-responsive esophageal eosinophilia but not eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2014; **12**: 1815-1823.e2 [PMID: 24657840 DOI: 10.1016/j.cgh.2014.02.037]

P- Reviewer: Abdel-Hamid SMM, Akiho H, Mann O, Venu RP
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ISSN 2150-5330 (online)

World Journal of *Gastrointestinal Pathophysiology*

World J Gastrointest Pathophysiol 2018 November 12; 9(4): 73-78

Published by Baishideng Publishing Group Inc

WJGP

World Journal of Gastrointestinal Pathophysiology

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Volume 9 Number 4 November 12, 2018

EDITORIAL

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World Journal of Gastrointestinal Pathophysiology
Volume 9 Number 4 November 12, 2018

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World Journal of Gastrointestinal Pathophysiology is now abstracted and indexed in Emerging Sources Citation Index (Web of Science), PubMed, PubMed Central, China National Knowledge Infrastructure (CNKI), and Superstar Journals Database.

NAME OF JOURNAL
World Journal of Gastrointestinal Pathophysiology

ISSN
ISSN 2150-5330 (online)

LAUNCH DATE
April 15, 2010

World Journal of Gastrointestinal Pathophysiology
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
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E-mail: editorialoffice@wjgnet.com
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PUBLICATION DATE
November 12, 2018

INSTRUCTIONS TO AUTHORS
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World Journal of Gastrointestinal Pathophysiology

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World J Gastrointest Pathophysiol 2018 November 12; 9(4): 73-78

DOI: 10.4291/wjgp.v9.i4.73

ISSN 2150-5330 (online)

EDITORIAL

Role of *TNFSF15* in the intestinal inflammatory response

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Author contributions: All authors contributed equally to the research for this manuscript; Tourtourikov I and Kadiyska T approved the final version of the article.

Conflict-of-interest statement: The authors have no conflict of interest to declare.

Manuscript source: Invited manuscript

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Received: August 3, 2018
Peer-review started: August 3, 2018
First decision: August 30, 2018
Revised: October 11, 2018
Accepted: October 17, 2018
Article in press: October 18, 2018
Published online: November 12, 2018

Abstract

Gastrointestinal diseases, specifically Crohn's disease, ulcerative colitis, diverticular disease, and primary biliary cirrhosis are all characterized by complicated inflammation of the digestive tract. Their pathology is multifactorial, and risk factors encompass both genetic and environmental factors. Recent advances in the genetic component of inflammatory bowel diseases (IBDs) have revealed that the tumor necrosis factor superfamily member 15 (*TNFSF15*) contains a number of risk alleles associated not only with IBD but also with other diseases such as diverticular disease and primary biliary cirrhosis. These risk alleles in *TNFSF15* and the altered expression of its gene product can serve as the common ground between these disorders by explaining at least some of the underlying processes that lead to a dysregulated immune response and subsequent chronic inflammation. Here, we aim to outline how the *TNFSF15* gene is involved in the proliferation and cell fate of different populations of T cells and subsequently in the control of both pro- and anti-inflammatory cytokines. Furthermore, we summarize what is currently known of *TNFSF15* control region variants, how they are associated with each mentioned disease, and how these variants can explain the autoimmune pathology of said diseases through altered *TNFSF15* expression.

Key words: Tumor necrosis factor superfamily member 15; Diverticular disease; Death receptor 3; Ulcerative colitis; Crohn's disease; Primary biliary cirrhosis

Core tip: Tumor necrosis factor superfamily member 15 and the protein it encodes, tumor necrosis factor ligand-related molecule 1 play a vital role in the mucosal immunity. Expression of tumor necrosis factor ligand-related molecule 1 and death receptor 3-mediated signaling both exert their effects in Crohn's disease, ulcerative colitis, diverticular disease, and primary biliary cirrhosis, which can serve to bridge the gap of knowledge regarding the genetic components of this group of inflammatory diseases as well as provide common ground for a putative targeted treatment.

Kadiyska T, Tourtourikov I, Popmihaylova AM, Kadian H, Chavoushian A. Role of *TNFSF15* in the intestinal inflammatory response. *World J Gastrointest Pathophysiol* 2018; 9(4): 73-78 Available from: URL: http://www.wjgnet.com/2150-5330/full/v9/i4/73.htm DOI: http://dx.doi.org/10.4291/wjgp.v9.i4.73

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic disorder that constitutes an important worldwide health problem. This group of diseases is multifactorial and characterized by chronic relapsing intestinal inflammation[1]. The two major subtypes of IBD are ulcerative colitis (UC) and Crohn's disease (CD). IBD is a global disease with the highest prevalence in Western countries (North America, Europe, and Oceania) although recently there has been an accelerated incidence rate in the newly industrialized countries of Asia, South America, and Africa, where societies have become more westernized[1]. Although the exact etiology of IBDs is still unknown, numerous studies have revealed the multifactorial nature of IBDs that encompasses genetic susceptibility, environmental factors, intestinal microbiota, and the immune response system[2].

Another common gastrointestinal disorder, similar in its prevalence amongst western populations is colonic diverticulosis. The term "diverticulosis" refers to the occurrence of diverticula due to the formation of pouches by the mucosal wall of the intestine[3]. Colonic diverticulosis, or diverticular disease (DD), is a broad-spectrum term, as the condition involves a number of clinical manifestations that can range from the presence of constant abdominal systems without inflammation (symptomatic uncomplicated diverticular disease) to a significant and symptomatic inflammatory process (segmental colitis associated with diverticulosis and diverticulitis)[4].

Both diverticulitis and IBDs share overlapping characteristics and symptoms including, but not limited to: clinical presentation involving diarrhea, mucus in the stool, abdominal pain, weight loss, fistulae, bowel structuring, and inflammation[5,6]. This overlap can make diagnosis difficult for the attending clinician, although distinction can be achieved by endoscopical examination[5]. Despite this difficulty and in order to improve our understanding of the relation between inflammation and gastrointestinal disorders, we have to ask the question, what is the driving factor behind these shared attributes of CD, UC, and DD?

The common ground for the pathological signs of IBDs and DD appears to be a dysregulated mucosal immune response[7,8]. This dysregulation often results in impaired epithelial barrier function and damage to the surrounding epithelial tissue. Both pro- and anti-inflammatory cell lines and their respective secreted cytokines are involved in this response. In CD, T helper 1 (Th1)/Th17 cells and interleukin (IL)-12 as well as IL-23 are characteristic, whereas in UC the major factor is natural killer T (NKT) cells secreting IL-13 and IL-5[9]. Tumor necrosis factor superfamily member 15 (*TNFSF15*), also known as tumor necrosis factor ligand-related molecule 1 (TL1A) and vascular endothelial growth inhibitor (VEGI) is a tumor necrosis factor (TNF) family member encoding a ligand produced by a variety of cell lines, including endothelial cells, macrophages, dendritic cells (DCs), and T cells[10]. First described in 2002 as a T-cell stimulatory cytokine[11], studies have discovered that it affects cell lines related to both the innate and adaptive immune responses by its receptor, death receptor 3 (DR3)[12]. Since then, the role of this cytokine-receptor pair has been linked to the immunomodulation and vascular endothelial function observed in IBDs[6].

TNFSF15 FUNCTION AND EXPRESSION

The gene product of *TNFSF15*, TL1A, is a TNF-like factor, which is expressed in endothelial cells (human umbilical vein endothelial cells, adult dermal micro-vascular endothelial cells, and uterus myometrial endothelial cells), gut lamina propria lymphocytes, and macrophages.

TL1A is a longer splicing variant of the coding gene *TNFSF15* compared to the initially described protein TL1/VEGI. The difference between the two variants is that TL1A is encoded by all four coding exons, whereas a continuous DNA containing the fourth exon and its 5' adjacent intron encodes TL1. As a result, the two variants have identical C-terminal regions while the N-terminal regions are different for the two proteins. TL1A is a type II transmembrane protein, containing 251 amino acids and has a molecular weight of 28 kDa. The transmembrane form of TL1A can be cleaved by enzymes and exists as a functional soluble protein[11,13]. This cleavage can vary depending on the cell of origin[14]. The soluble form is more abundantly synthesized by DCs, and the membrane-bound protein is expressed by both T cells and DCs. The different forms, like other members of the TNF superfamily, have different functions. Soluble TL1A can be detected after DC and monocyte stimulation *in vitro*, and increased levels have been detected in serum samples from patients with rheumatoid arthritis, an autoreactive disease[15].

The receptor for TL1A, DR3, was identified in the

1990s[16], and was later discovered to be highly homologous to TNF receptor 1 (TNFR1)[12]. Signaling by DR3 is facilitated primarily through the use of TNFR-associated death domain protein, which contains a TNF receptor associated factors-binding domain as well as a death domain. This combination allows DR3 to activate nuclear factor kappa B and mitogen-activated protein kinase[17], which allows it to play a role in both apoptosis and anti-apoptosis, cell survival, and proliferation[18].

The expression of TL1A is closely linked to the levels of inflammation over the course of IBD and is also correlated to areas affected by the disease[10]. While TL1A baseline expression can be low[19], pro-inflammatory stimulation seems to be the switch that increases TL1A expression[20]. Both TL1A and DR3 are expressed across all members of the T cell family[21,22], despite the original discovery of TL1A in endothelial cells. The action of TL1A-DR3 signaling is most profound in the differentiation and stimulation of T cell subtypes. Co-stimulation with TL1A increases IL-2 signaling[11,20,23], whereas TL1A itself stimulates the proliferation of T cells. Specific $CD4^+$ T cells can up-regulate DR3 and produce interferon gamma in response to TL1A combined with IL-12 and IL-18 in the intestinal mucosa[23], suggesting a putative mechanism for TL1A gut signaling and expression[19]. TL1A also affects Th17 cells as DR3 expression is highly upregulated on this cell subset[24], although TL1A-mediated Th17 proliferation is achieved in a DR-3 independent manner[20]. Furthermore, TL1A plays a role in the development of regulatory T cells (T_{reg}), as stimulation by the soluble form of TL1A increases T_{reg} proliferation[25]. However, *in vitro* assays have shown that the increased numbers of T_{regs} also show reduced suppressive capability[26].

TNFSF15 AND INFLAMMATION

Genetic studies attempting to evaluate the role of *TNFSF15* have only begun recently following previously suggested hints of genetic factors involved in IBD[27,28]. The first genome-wide association studies (GWAS) conducted in 2005 discovered an association between TL1A and CD in a Japanese cohort of patients[29]. Subsequent studies have replicated and confirmed the association of TNFSF15 in European populations, for patients with both CD or UC[30]. Further investigation on specific patient subsets confirmed the protective haplotype[31] and revealed that TL1A expression is increased in carriers of the risk haplotype in a Jewish cohort of patients with CD and *Escherichia coli* exposure[32].

The findings of the previously mentioned studies and the data obtained allowed for the further investigation of *TNFSF15* single nucleotide polymorphisms (SNPs) and their role not only in CD and UC, but also in DD. The first case study aiming to investigate how these SNPs can exert an effect revealed that the SNP rs7848647 and specifically, the risk allele G conferred an additive higher risk towards DD requiring surgical intervention[33]. As a follow-up, another study aimed to increase the number of participants and to include six other SNPs, four of which had been previously associated with CD[29] as a risk haplotype and to reveal if such an association could be found for DD as well. Results demonstrated not only that the CD risk haplotype was associated with DD, but two protective haplotypes emerged as well[6]. Although both studies provided hopeful results, they also suggest that there might be further undiscovered SNPs in DD pathology.

TNFSF15 variants have also been associated with primary biliary cirrhosis (PBC), a chronic and progressive liver disease, leading to hepatic failure and liver cirrhosis. One of the hallmarks of PBC is an autoimmune reaction towards biliary epithelial cells. Combined with data from twin studies, this has driven research into a possible genetic component of PBC. The first GWAS studies demonstrated the association of *TNFSF15* rs4979462 in Asian populations with PBC[34] as the second strongest susceptibility gene. Specifically, rs4979462 has been found to be one of the main causal variants in the gene, due to a creation of a novel nuclear factor 1 binding site, a finding further strengthened by the increased *TNFSF15* mRNA expression[35]. Other large-scale GWAS studies have also demonstrated that *TNFSF15* is a part of a multitude of PBC risk loci involved in T cell, B cell, and natural killer (NK) cell stimulation and proliferation[36].

TL1A expression has also proven to be one of the key hallmarks of IBD. It was first observed in CD and UC[10,21,37] with both increased protein and mRNA levels compared to healthy controls[19]. This expression is regulated in a two-fold manner in gastrointestinal disorders. First, single-nucleotide polymorphisms have been found to correlate with TL1A expression[32,38,39] in a variety of immune cells. Second, TL1A can be induced by a number of gut-specific bacteria[22] with expression levels adjusting accordingly to the presence or absence of bacteria. Localized increased inflammation correlating with increased TL1A expression and exposure to bacteria in patients with CD, UC, and DD appears to be the common ground between these gastrointestinal disorders (Figure 1).

Further proof for the involvement of *TNFSF15* comes in the form of studies investigating the association of risk variants within the gene and the requirement of surgical intervention as part of the treatment plan[33,40]. Cases that do not respond to medical treatment and present themselves with severe colonic inflammation ultimately require surgical resection, which represents a higher risk for the patient.

Because of its mode of action, and by having a very specific niche of activity and expression, *TNFSF15* can be considered a putative therapeutic target. Studies have investigated the effect of anti-TL1A antibodies on sodium-sulfate induced colitis[41] and T-cell transfer models[42]. Some have even successfully managed to reverse fibrosis in these models[43]. As TL1A expression can vary, depending on genetic variations in its control

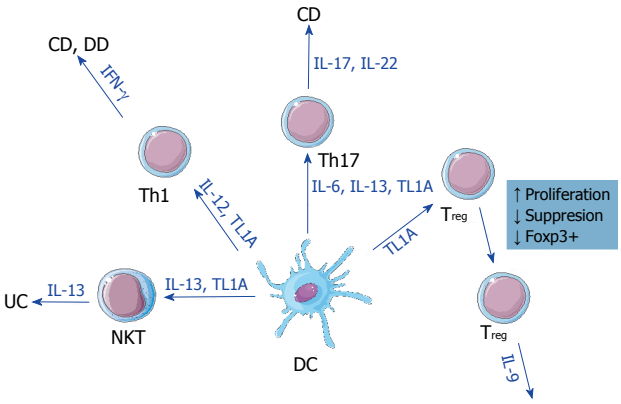

Figure 1 Effect of tumor necrosis factor ligand-related molecule 1 (TL1A) signaling across different subtypes of leukocytes. TL1A acts by promoting cytokine signaling, cell differentiation, and proliferation. These effects vary between different subtypes. While TL1A promotes natural killer T cells and T helper Th1/Th17 proliferation, it can also suppress regulatory T cell functions and forkhead box P3 positive expression. Regardless, TL1A is not associated exclusively with any form. CD: Crohn's disease; DC: Dendritic cell; DD: Diverticular disease; IFN-γ: Interferon gamma; IL: Interleukin; NKT: Natural killer T cells; TL1A: Tumor necrosis factor Ligand-related molecule 1; Th: T helper; T_{reg}: Regulatory T cell; Foxp3: Forkhead box P3; UC: Ulcerative colitis.

region, it remains to be seen whether a targeted anti-TL1A therapy can be applied in a clinical setting.

CONCLUSION

TNFSF15 and the protein it encodes share a unique position amongst other genetic factors for gastrointestinal disorders. It enhances the T cell responses in CD, UC, and DD acting as a bridge between these disorders. It also plays a role in the immune response of mucosal tissue against bacterial infections, another common factor in gastrointestinal disorders. Furthermore, genetic variations in this gene have been associated as risk factors for all of the diseases examined here. All of these characteristics point towards the further study of *TNFSF15* and the TL1A-DR3 interaction among patients with IBD and DD, both as a putative therapeutic target and a risk prediction factor.

REFERENCES

1 **Ng SC,** Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, Panaccione R, Ghosh S, Wu JCY, Chan FKL, Sung JJY, Kaplan GG. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet* 2017; **390**: 2769-2778 [DOI: 10.1016/S0140-6736(17)32448-0]

2 **Kamm MA**. Rapid changes in epidemiology of inflammatory bowel disease. *Lancet* 2017; **390**: 2741-2742 [DOI: 10.1016/S0140-6736(17)32669-7]

3 **Ceresoli M**, Lo Bianco G, Gianotti L, Nespoli L. Inflammation management in acute diverticulitis: current perspectives. *J Inflamm Res* 2018; **11**: 239-246 [PMID: 29881303 DOI: 10.2147/JIR.S142990]

4 **Strate LL**, Modi R, Cohen E, Spiegel BM. Diverticular disease as a chronic illness: evolving epidemiologic and clinical insights. *Am J Gastroenterol* 2012; **107**: 1486-1493 [PMID: 22777341 DOI: 10.1038/ajg.2012.194]

5 **Peppercorn MA**. The overlap of inflammatory bowel disease and diverticular disease. *J Clin Gastroenterol* 2004; **38**: S8-10 [PMID: 15115922 DOI: 10.1097/01.mcg.0000123993.13937.ec]

6 **Connelly TM**, Choi CS, Berg AS, Harris L 3rd, Coble J, Koltun WA. Diverticulitis and Crohn's disease have distinct but overlapping tumor necrosis superfamily 15 haplotypes. *J Surg Res* 2017; **214**: 262-269 [PMID: 28624054 DOI: 10.1016/j.jss.2017.02.030]

7 **Tursi A**. Diverticular disease: A therapeutic overview. *World J Gastrointest Pharmacol Ther* 2010; **1**: 27-35 [PMID: 21577292 DOI: 10.4292/wjgpt.v1.i1.27]

8 **Xu XR**, Liu CQ, Feng BS, Liu ZJ. Dysregulation of mucosal immune response in pathogenesis of inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 3255-3264 [PMID: 24695798 DOI: 10.3748/wjg.v20.i12.3255]

9 **Strober W**, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 2011; **140**: 1756-1767 [PMID: 21530742 DOI: 10.1053/j.gastro.2011.02.016]

10 **Bamias G**, Martin C 3rd, Marini M, Hoang S, Mishina M, Ross WG, Sachedina MA, Friel CM, Mize J, Bickston SJ, Pizarro TT, Wei P, Cominelli F. Expression, localization, and functional activity of TL1A, a novel Th1-polarizing cytokine in inflammatory bowel disease. *J Immunol* 2003; **171**: 4868-4874 [PMID: 14568967 DOI: 10.4049/jimmunol.171.9.4868]

11 **Migone TS**, Zhang J, Luo X, Zhuang L, Chen C, Hu B, Hong JS, Perry JW, Chen SF, Zhou JX, Cho YH, Ullrich S, Kanakaraj P, Carrell J, Boyd E, Olsen HS, Hu G, Pukac L, Liu D, Ni J, Kim S, Gentz R, Feng P, Moore PA, Ruben SM, Wei P. TL1A is a TNF-like ligand for DR3 and TR6/DcR3 and functions as a T cell costimulator. *Immunity* 2002; **16**: 479-492 [PMID: 11911831 DOI: 10.1016/S1074-7613(02)00283-2]

12 **Chinnaiyan AM**, O'Rourke K, Yu GL, Lyons RH, Garg M, Duan DR, Xing L, Gentz R, Ni J, Dixit VM. Signal transduction by DR3, a death domain-containing receptor related to TNFR-1 and CD95. *Science* 1996; **274**: 990-992 [PMID: 8875942 DOI: 10.1126/science.274.5289.990]

13 **Zhan C**, Yan Q, Patskovsky Y, Li Z, Toro R, Meyer A, Cheng H, Brenowitz M, Nathenson SG, Almo SC. Biochemical and structural characterization of the human TL1A ectodomain. *Biochemistry* 2009; **48**: 7636-7645 [PMID: 19522538 DOI: 10.1021/bi900031w]

14 **Ferdinand JR**, Richard AC, Meylan F, Al-Shamkhani A, Siegel RM. Cleavage of TL1A Differentially Regulates Its Effects on Innate and Adaptive Immune Cells. *J Immunol* 2018; **200**: 1360-1369 [PMID: 29335258 DOI: 10.4049/jimmunol.1700891]

15 **Siakavellas SI**, Sfikakis PP, Bamias G. The TL1A/DR3/DcR3 pathway in autoimmune rheumatic diseases. *Semin Arthritis Rheum* 2015; **45**: 1-8 [PMID: 25887448 DOI: 10.1016/j.semarthrit.2015.02.007]

16 **Screaton GR**, Xu XN, Olsen AL, Cowper AE, Tan R, McMichael AJ, Bell JI. LARD: a new lymphoid-specific death domain containing receptor regulated by alternative pre-mRNA splicing. *Proc Natl Acad Sci USA* 1997; **94**: 4615-4619 [PMID: 9114039 DOI: 10.1073/pnas.94.9.4615]

17 **Wen L**, Zhuang L, Luo X, Wei P. TL1A-induced NF-kappaB activation and c-IAP2 production prevent DR3-mediated apoptosis in TF-1 cells. *J Biol Chem* 2003; **278**: 39251-39258 [PMID: 12882979 DOI: 10.1074/jbc.M305833200]

18 **Gaur U**, Aggarwal BB. Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. *Biochem Pharmacol* 2003; **66**: 1403-1408 [PMID: 14555214 DOI: 10.1016/S0006-2952(03)00490-8]

19 **Richard AC**, Ferdinand JR, Meylan F, Hayes ET, Gabay O, Siegel RM. The TNF-family cytokine TL1A: from lymphocyte costimulator to disease co-conspirator. *J Leukoc Biol* 2015; **98**: 333-345 [PMID: 26188076 DOI: 10.1189/jlb.3RI0315-095R]

20 **Meylan F**, Davidson TS, Kahle E, Kinder M, Acharya K, Jankovic D, Bundoc V, Hodges M, Shevach EM, Keane-Myers A, Wang EC, Siegel RM. The TNF-family receptor DR3 is essential for diverse T cell-mediated inflammatory diseases. *Immunity* 2008; **29**: 79-89 [PMID: 18571443 DOI: 10.1016/j.immuni.2008.04.021]

21 **Prehn JL**, Thomas LS, Landers CJ, Yu QT, Michelsen KS, Targan SR. The T cell costimulator TL1A is induced by FcgammaR signaling in human monocytes and dendritic cells. *J Immunol* 2007; **178**: 4033-4038 [PMID: 17371957 DOI: 10.4049/jimmunol.178.7.4033]

22 **Shih DQ**, Kwan LY, Chavez V, Cohavy O, Gonsky R, Chang EY, Chang C, Elson CO, Targan SR. Microbial induction of inflammatory bowel disease associated gene TL1A (TNFSF15) in antigen presenting cells. *Eur J Immunol* 2009; **39**: 3239-3250 [PMID: 19839006 DOI: 10.1002/eji.200839087]

23 **Jin S**, Chin J, Seeber S, Niewoehner J, Weiser B, Beaucamp N, Woods J, Murphy C, Fanning A, Shanahan F, Nally K, Kajekar R, Salas A, Planell N, Lozano J, Panes J, Parmar H, DeMartino J, Narula S, Thomas-Karyat DA. TL1A/TNFSF15 directly induces proinflammatory cytokines, including TNFα, from CD3+CD161+ T cells to exacerbate gut inflammation. *Mucosal Immunol* 2013; **6**: 886-899 [PMID: 23250276 DOI: 10.1038/mi.2012.124]

24 **Pappu BP**, Borodovsky A, Zheng TS, Yang X, Wu P, Dong X, Weng S, Browning B, Scott ML, Ma L, Su L, Tian Q, Schneider P, Flavell RA, Dong C, Burkly LC. TL1A-DR3 interaction regulates Th17 cell function and Th17-mediated autoimmune disease. *J Exp Med* 2008; **205**: 1049-1062 [PMID: 18411337 DOI: 10.1084/jem.20071364]

25 **Schreiber TH**, Wolf D, Tsai MS, Chirinos J, Deyev VV, Gonzalez L, Malek TR, Levy RB, Podack ER. Therapeutic Treg expansion in mice by TNFRSF25 prevents allergic lung inflammation. *J Clin Invest* 2010; **120**: 3629-3640 [PMID: 20890040 DOI: 10.1172/JCI42933]

26 **Taraban VY**, Slebioda TJ, Willoughby JE, Buchan SL, James S, Sheth B, Smyth NR, Thomas GJ, Wang EC, Al-Shamkhani A. Sustained TL1A expression modulates effector and regulatory T-cell responses and drives intestinal goblet cell hyperplasia. *Mucosal Immunol* 2011; **4**: 186-196 [PMID: 20962771 DOI: 10.1038/mi.2010.70]

27 **Bonen DK**, Cho JH. The genetics of inflammatory bowel disease. *Gastroenterology* 2003; **124**: 521-536 [PMID: 12557156 DOI: 10.1053/gast.2003.50045]

28 **Brant SR**, Shugart YY. Inflammatory bowel disease gene hunting by linkage analysis: rationale, methodology, and present status of the field. *Inflamm Bowel Dis* 2004; **10**: 300-311 [PMID: 15290927 DOI: 10.1097/00054725-200405000-00019]

29 **Yamazaki K**, McGovern D, Ragoussis J, Paolucci M, Butler H, Jewell D, Cardon L, Takazoe M, Tanaka T, Ichimori T, Saito S, Sekine A, Iida A, Takahashi A, Tsunoda T, Lathrop M, Nakamura Y. Single nucleotide polymorphisms in TNFSF15 confer susceptibility to Crohn's disease. *Hum Mol Genet* 2005; **14**: 3499-3506 [PMID: 16221758 DOI: 10.1093/hmg/ddi379]

30 **Jostins L**, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, Lee JC, Schumm LP, Sharma Y, Anderson CA, Essers J, Mitrovic M, Ning K, Cleynen I, Theatre E, Spain SL, Raychaudhuri S, Goyette P, Wei Z, Abraham C, Achkar JP, Ahmad T, Amininejad L, Ananthakrishnan AN, Andersen V, Andrews JM, Baidoo L, Balschun T, Bampton PA, Bitton A, Boucher G, Brand S, Büning C, Cohain A, Cichon S, D'Amato M, De Jong D, Devaney KL, Dubinsky M, Edwards C, Ellinghaus D, Ferguson LR, Franchimont D, Fransen K, Gearry R, Georges M, Gieger C, Glas J, Haritunians T, Hart A, Hawkey C, Hedl M, Hu X, Karlsen TH, Kupcinskas L, Kugathasan S, Latiano A, Laukens D, Lawrance IC, Lees CW, Louis E, Mahy G, Mansfield J, Morgan AR, Mowat C, Newman W, Palmieri O, Ponsioen CY, Potocnik U, Prescott NJ, Regueiro M, Rotter JI, Russell RK, Sanderson JD, Sans M, Satsangi J, Schreiber S, Simms LA, Sventoraityte J, Targan SR, Taylor KD, Tremelling M, Verspaget HW, De Vos M, Wijmenga C, Wilson DC, Winkelmann J, Xavier RJ, Zeissig S, Zhang B, Zhang CK, Zhao H; International IBD Genetics Consortium (IIBDGC), Silverberg MS, Annese V, Hakonarson H, Brant SR, Radford-Smith G, Mathew CG, Rioux JD, Schadt EE, Daly MJ, Franke A, Parkes M, Vermeire S, Barrett JC, Cho JH. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012; **491**: 119-124 [PMID: 23128233 DOI: 10.1038/nature11582]

31 **Picornell Y**, Mei L, Taylor K, Yang H, Targan SR, Rotter JI. TNFSF15 is an ethnic-specific IBD gene. *Inflamm Bowel Dis* 2007; **13**: 1333-1338 [PMID: 17663424 DOI: 10.1002/ibd.20223]

32 **Michelsen KS**, Thomas LS, Taylor KD, Yu QT, Mei L, Landers CJ, Derkowski C, McGovern DP, Rotter JI, Targan SR. IBD-associated TL1A gene (TNFSF15) haplotypes determine increased expression of TL1A protein. *PLoS One* 2009; **4**: e4719 [PMID: 19262684 DOI: 10.1371/journal.pone.0004719]

33 **Connelly TM**, Berg AS, Hegarty JP, Deiling S, Brinton D, Poritz LS, Koltun WA. The TNFSF15 gene single nucleotide polymorphism rs7848647 is associated with surgical diverticulitis. *Ann Surg* 2014; **259**: 1132-1137 [PMID: 24814505 DOI: 10.1097/SLA.0000000000000232]

34 **Nakamura M**, Nishida N, Kawashima M, Aiba Y, Tanaka A, Yasunami M, Nakamura H, Komori A, Nakamuta M, Zeniya M, Hashimoto E, Ohira H, Yamamoto K, Onji M, Kaneko S, Honda M, Yamagiwa S, Nakao K, Ichida T, Takikawa H, Seike M, Umemura T, Ueno Y, Sakisaka S, Kikuchi K, Ebinuma H, Yamashiki N, Tamura S, Sugawara Y, Mori A, Yagi S, Shirabe K, Taketomi A, Arai K, Monoe K, Ichikawa T, Taniai M, Miyake Y, Kumagi T, Abe M, Yoshizawa K, Joshita S, Shimoda S, Honda K, Takahashi H, Hirano K, Takeyama Y, Harada K, Migita K, Ito M, Yatsuhashi H, Fukushima N, Ota H, Komatsu T, Saoshiro T, Ishida J, Kouno H, Kouno H, Yagura M, Kobayashi M, Muro T, Masaki N, Hirata K, Watanabe Y, Nakamura Y, Shimada M, Hirashima N, Komeda T, Sugi K, Koga M, Ario K, Takesaki E, Maehara Y, Uemoto S, Kokudo N, Tsubouchi H, Mizokami M, Nakanuma Y, Tokunaga K, Ishibashi H. Genome-wide association study identifies TNFSF15 and POU2AF1 as susceptibility loci for primary biliary cirrhosis in the Japanese population. *Am J Hum Genet* 2012; **91**: 721-728 [PMID: 23000144 DOI: 10.1016/j.ajhg.2012.08.010]

35 **Hitomi Y**, Kawashima M, Aiba Y, Nishida N, Matsuhashi M, Okazaki H, Nakamura M, Tokunaga K. Human primary biliary cirrhosis-susceptible allele of rs4979462 enhances TNFSF15 expression by binding NF-1. *Hum Genet* 2015; **134**: 737-747 [PMID: 25899471 DOI: 10.1007/s00439-015-1556-3]

36 **Qiu F**, Tang R, Zuo X, Shi X, Wei Y, Zheng X, Dai Y, Gong Y, Wang L, Xu P, Zhu X, Wu J, Han C, Gao Y, Zhang K, Jiang Y, Zhou J, Shao Y, Hu Z, Tian Y, Zhang H, Dai N, Liu L, Wu X, Zhao W, Zhang X, Zang Z, Nie J, Sun W, Zhao Y, Mao Y, Jiang P, Ji H, Dong Q, Li J, Li Z, Bai X, Li L, Lin M, Dong M, Li J, Zhu P, Wang C, Zhang Y, Jiang P, Wang Y, Jawed R, Xu J, Zhang Y, Wang Q, Yang Y, Yang F, Lian M, Jiang X, Xiao X, Li Y, Fang J, Qiu D, Zhu Z, Qiu H, Zhang J, Tian W, Chen S, Jiang L, Ji B, Li P, Chen G, Wu T, Sun Y, Yu J, Tang H, He M, Xia M, Pei H, Huang L, Qing Z, Wu J, Huang Q, Han J, Xie W, Sun Z, Guo J, He G, Eric Gershwin M, Lian Z, Liu X, Seldin MF, Liu X, Chen W, Ma X. A genome-wide association study identifies six novel risk loci for primary biliary cholangitis. *Nat Commun* 2017; **8**: 14828 [PMID: 28425483 DOI: 10.1038/ncomms14828]

37 **Kamada N**, Hisamatsu T, Honda H, Kobayashi T, Chinen H, Takayama T, Kitazume MT, Okamoto S, Koganei K, Sugita A, Kanai T, Hibi T. TL1A produced by lamina propria macrophages induces Th1 and Th17 immune responses in cooperation with IL-23 in patients with Crohn's disease. *Inflamm Bowel Dis* 2010; **16**: 568-575 [PMID: 19834969 DOI: 10.1002/ibd.21124]

38 **Hedl M**, Abraham C. A TNFSF15 disease-risk polymorphism increases pattern-recognition receptor-induced signaling through caspase-8-induced IL-1. *Proc Natl Acad Sci USA* 2014; **111**: 13451-13456 [PMID: 25197060 DOI: 10.1073/pnas.1404178111]

39 **Kakuta Y**, Ueki N, Kinouchi Y, Negoro K, Endo K, Nomura E, Takagi S, Takahashi S, Shimosegawa T. TNFSF15 transcripts from risk haplotype for Crohn's disease are overexpressed in stimulated T cells. *Hum Mol Genet* 2009; **18**: 1089-1098 [PMID: 19124533 DOI: 10.1093/hmg/ddp005]

40 **Haritunians T**, Taylor KD, Targan SR, Dubinsky M, Ippoliti A, Kwon S, Guo X, Melmed GY, Berel D, Mengesha E, Psaty BM, Glazer NL, Vasiliauskas EA, Rotter JI, Fleshner PR, McGovern DP. Genetic predictors of medically refractory ulcerative colitis. *Inflamm Bowel Dis* 2010; **16**: 1830-1840 [PMID: 20848476 DOI: 10.1002/ibd.21293]

41 **Takedatsu H**, Michelsen KS, Wei B, Landers CJ, Thomas LS,

Dhall D, Braun J, Targan SR. TL1A (TNFSF15) regulates the development of chronic colitis by modulating both T-helper 1 and T-helper 17 activation. *Gastroenterology*2008; **135**: 552-567 [PMID: 18598698 DOI: 10.1053/j.gastro.2008.04.037]

42 **Zheng L**, Zhang X, Chen J, Ichikawa R, Wallace K, Pothoulakis C, Koon HW, Targan SR, Shih DQ. sustained tlla *(tnfsf15)* expression on both lymphoid and myeloid cells leads to mild spontaneous intestinal inflammation and fibrosis. *Eur J Microbiol Immunol (Bp)* 2013; **3**: 11-20 [PMID: 23638306 DOI: 10.1556/EuJMI.3.2013.1.2]

43 **Shih DQ**, Zheng L, Zhang X, Zhang H, Kanazawa Y, Ichikawa R, Wallace KL, Chen J, Pothoulakis C, Koon HW, Targan SR. Inhibition of a novel fibrogenic factor Tl1a reverses established colonic fibrosis. *Mucosal Immunol* 2014; **7**: 1492-1503 [PMID: 24850426 DOI: 10.1038/mi.2014.37]

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